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ACTA CYTOLOGICA

THE JOURNAL OF EXFOLIATIVE CYTOLOGY

The Official Periodical of
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Requests for subscriptions should be sent to the Editorial Office. Checks should be made payable to ACTA CYTOLOGICA, Volumes I, II, and III are still available. The current Volume IV will be issued within the calendar year 1960. There will be a change in the format of the Journal and the frequency of its publication with the following Volume V, scheduled to begin in January, 1961.

In Memoriam

Walter Schiller

1887-1960

Walter Schiller was born in Vienna, Austria, in the year 1887, and graduated from Medical Faculty of Vienna University in the year 1912. After his four years of military service during World War I, he was, for a period of three years, with the Department of Pathology of the University and for three years with the First Department of Medicine of the University Clinics.

In 1921, Dr. Schiller became associated with the Second University Department of Obstetrics and Gynecology (the former Wertheim Clinic) where he was in charge of the Pathology Laboratory of the Department. He was in this position until the year 1937, when he moved to the United States of America where he found his second home in Chicago. There he was first with the Cook County Hospital of Chicago, and later with the State Roseland Hospital and the Women's and Children's Hospital of Chicago.

His most known publications were written when he was in the Second University Department of Obstetrics and Gynecology in Vienna, Austria. Among them are the papers on the early diagnosis of the cervical carcinoma. His test with Lugol Iodine and the technique of scraping the ectocervical epithelium with the sharp spoon received world wide attention. Together with Robert Meyer, he was one of the first who postulated and found recognition for the diagnosis of carcinoma in situ, the pre-invasive lesion. In addition to these works, Walter Schiller was also the author of numerous publications concerning other fields of gynecology. His second field of interest in addition to cervical carcinoma was the ovarian tumors, and he published a monograph on granulosa cell tumors. He was one of the Founding Members of the American College of Pathologists, and he was an Honorary Member of the German Society of Physicians and of the Austrian Society of Gynecology.

The curriculum vitae of Walter Schiller would be incomplete if one did not mention his other universal abilities. He had a scholarly knowledge of mathematics and the natural sciences as well as of literature, evidenced by his extraordinarily rich and well selected library. He belongs to the group of few men who are not only well versed in their own specialties, but also in most any other field of knowledge. He was a real polyhistor. This made his fascinating personality. His lively and critical mind was with him during the many years of his illness, making him the living example of the victory of the mind over the body.

TASSILO ANTOINE

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ACADEMIE INTERNATIONALE DE CYTOLOGIE GYNECOLOGIQUE
INTERNATIONALE AKADEMIE FÜR GYNÄKOLOGISCHE ZYTOLOGIE
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BUSINESS MATTERS
OF THE INTERNATIONAL ACADEMY

FROM THE OFFICE OF THE PRESIDENT

In accordance with the Bylaws of the International Academy Article I, Section 3, the

FIRST INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY

is scheduled for August 31 to September 2, 1961, immediately preceding the Third World Gynecology Congress. The place of the meeting will be Vienna, Austria.

Extracts from the Bylaws concerning the Scientific Session (*Article 1, Section 10*) : ". . . Papers presented at the Scientific Session shall be original papers which have never been presented or published. Material which has already been published or presented elsewhere may be considered in panel discussions. Fifty per cent of the Scientific Session shall be devoted to original papers, and fifty per cent of the time to panel discussions. . . ."

The attendance and participation is *not* restricted to members of the International Academy.

The Congress of Exfoliative Cytology will deal with, among other general topics, the following two main subjects:

(1) Progression and regression of epithelial abnormalities, and

(2) The hormonal activity as reflected in the vaginal smears in cases of gynecological tumors, breast tumors, and ovarian dystrophy.

In addition to other panel discussions, there will be an extensive discussion on cytological and histological terminologies and definitions.

Application forms for participants may be obtained from

The Offiice of the Secretary
International Congress of Exfoliative Cytology
666 Elm Street
Buffalo 3, New York, U.S.A.

WANTED OR AVAILABLE

It is the purpose of this column to promote international exchange of cytologists and cytotechnicians, to inform them of open permanent positions, and to inform employers of available cytology personnel. Persons interested in obtaining permanent positions as cytologists or cytotechnicians or in obtaining temporary fellowships in cytology (teaching, exchange, or training fellowships), and individuals or institutions offering such positions or openings are invited to write giving full information to: ACTA CYTOLOGICA, 5841 Maryland Avenue, Chicago 37, Illinois, U.S.A. Information supplied will be held strictly confidential.

While information received is subject to editing so that it conforms to the style of ACTA CYTOLOGICA, ACTA CYTOLOGICA cannot and do not assume responsibility for statements made by contributors.

OFFRES ET DEMANDES

Cette rubrique est destinée à favoriser l'échange international de cytologistes et de techniciens en cytologie. Elle renseignera sur les places permanentes vacantes et informera également sur le personnel cytologique disponible. Les personnes désirant obtenir une place permanente de cytologiste ou cytotechnicien, ou faire un stage temporaire en cytologie (enseignement, échange, training), et les instituts ou personnes offrant de telles places sont invités à écrire aux ACTA CYTOLOGICA (5841, Maryland Avenue, Chicago 37, Illinois, U.S.A.) en donnant tous les détails. Les informations reçues auront un caractère strictement confidentiel.

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Mit dieser Rubrik soll internationaler Stellenaustausch und Stellenvermittlung für Zytologen und zytologisch-technische Assistenten angebahnt werden, indem über offene Stellungen und über verfügbares Personal berichtet wird. Zytologen und zytologisch-technische Assistenten und Assistentinnen, die an vorübergehenden (Lehrstellen, Austauschstellen, Lernstellen) oder dauernden Anstellungen interessiert sind, und Personen oder Institutionen, die derartige Stellunge zu vergeben haben, sind gebeten an ACTA CYTOLOGICA (5841 South Maryland Avenue, Chicago 37, Illinois, U.S.A.) zu schreiben und möglichst genaue Einzelheiten anzugeben. Die erhaltenen Auskünfte und Einzelheiten werden streng vertraulich behandelt.

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El propósito de esta sección es promover el intercambio internacional de citólogos y técnicos en Citología, informar de vacantes en puestos permanentes, y de personal citológico disponible. Las personas que estén interesadas en obtener becas temporales, o puestos permanentes como citólogos o técnicos en Citología (Enseñanza, Intercambio, Becas de aprendizaje), y, asimismo, las personas o instituciones que puedan ofrecer tales puestos, deben escribir a ACTA CYTOLOGICA (5841 Maryland Avenue, Chicago 37, Illinois, USA) aportando información completa. Esta información será estrictamente confidencial.

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CYTOLOGISTS AND CYTOTECHNOLOGISTS WANTED

QUALIFIED CYTOTECHNOLOGIST WANTED full-time for Department of Pathology of Medical College of Georgia. Opportunities for research and teaching in medical school environment. New laboratories, well-equipped and air-conditioned. Attractive salary.
Please write to:
L. D. Stoddard, M.D., Professory of Pathology
Medical College of Georgia
Augusta, Georgia, U.S.A.

CYTOTECHNOLOGIST OR REGISTERED MEDICAL TECHNOLOGIST WANTED
250-bed general hospital, in a college community of approximately 4,000 people, wants cytotechnologist or registered medical technologist for its laboratory. 40-hour working week with occasional weekend duty. Residence for technicians available. Two weeks annual vacation plus two weeks sick leave. Salary for this position is open.
Please write to:
Dr. Elizabeth French, Director, Clinical Diagnostic Laboratories, Mary Hitchcock Memorial Hospital, 2 Maynard Street, Hanover, New Hampshire, U.S.A.

EXCHANGE CYTOTECHNOLOGIST WANTED: The central laboratory for Endocrinological Cytology and Cancer Cytology of the Health Insurance Institution Kupat-Holim, 34 Samenhof Street, Tel Aviv, Israel, is interested in an exchange program of cytotechnologists. The Central Laboratory is prepared to exchange, for a period of six months, one of its Senior Technologists, experienced in hormonal bioassays (F.S.H., estrogens), pregnancy tests, and endocrinological cytology, for a cytotechnologist who is experienced in cancer cytology and who is prepared to train laboratory staff members in Israel.
Please write to:
Dr. B. Bertinie, Head, Laboratory Division
The Central Laboratory for Endocrinological Cytology and Cancer Cytology of the Health Insurance Institution, Kupat-Holim, 34, Zamenhof Street,
Tel Aviv, Israel.

TRAINED CYTOTECHNOLOGIST IN EXFOLIATIVE CYTOLOGY WANTED
Cytotechnologist to work on a research project on fluorescence microscopy in cancer diagnosis. Living and working conditions in Winnipeg are very satisfactory. Depending on training and experience, the salary would range from $280 to $320 per month, the appointment being under grants from the National Cancer Institute of Canada and the Damon Runyon Fund.
Please write to:
Dr. Felix D. Bertalanffy, Department of Anatomy, University of Manitoba Medical College,
Bannatyne and Emily, Winnipeg 3, Manitoba, Canada

TRAINEES FOR CYTOTECHNOLOGY PROGRAM WANTED U. S. Public Health Service Fellowships available for training in Cytotechnology. Requirements are those of eligibility for certification in Cytotechnology by A.S.C.P. Duration—six months. Stipend $225.00 per month.
Please write to:
Peter A. Herbut, M.D., Professor of Pathology
Jefferson Medical College
Philadelphia, Pennsylvania, U.S.A.

TRAINEES FOR CYTOTECHNOLOGY PROGRAM WANTED U. S. Public Health Service Traineeships available for cytotechnology. Eligible are those who fulfill the basic requirements of the Board of Schools Medical Technology of the A.S.C.P. Tax free stipend $225.00 per month; duration not less than six months.
Please write to:
George L. Wied, M.D.
Assoc. Professor of Obstetrics and Gynecology
University of Chicago
Chicago 37, Illinois, U.S.A.

CYTOLOGISTS AND CYTOTECHNOLOGISTS AVAILABLE

MEDICAL TECHNOLOGIST, female, citizen of the United States of America, single

Cytology Training: Extensive training in anatomy, histology and cytology of female genital tract, hormonal evaluations, atypical cytology and carcinomas of the female genital tract with additional practical experience in the handling and processing of cytological material.

Wanted: European position as a gynecological cytotechnologist, beginning fall or winter - 1960.

Code No.: GAG 3/1/59, in care of ACTA CYTOLOGICA, 5841 S. Maryland Avenue, Chicago 37, Illinois, U.S.A.

TRAINED CYTOTECHNOLOGIST AVAILABLE FOR SCREENING Cytotechnologist with ten years of experience wants screening at home for pathologists and physicians. Will accept processed slides only. Charge per case.

Code No.: MIDDLEWEST 3/1/59, in care of ACTA CYTOLOGICA, 5841 S. Maryland Avenue, Chicago 37, Illinois, U. S. A.

CYTOLOGIST, M.D., male, permanent resident of U. S. A., originally from Italy.

Cytology Training: Twelve years training (two-year residency in Department of General Pathology in a Cancer Institute). Eight years as Chief Cytologist in Department of Public Health. Extensive University training in teaching and research. Knowledge of both cyto- and histochemistry.

Wanted: Appointment in a University Department or a Cancer Institute in the United States with a program in exfoliative cytology and opportunities for research.

Code No.: EDG 6/23/60, in care of ACTA CYTOLOGICA, 5841 S. Maryland Avenue, Chicago 37, Illinois, U. S. A.

TEACHING MATERIAL AVAILABLE

Laboratories doing exfoliative cytology in a general hospital often have difficulty maintaining their diagnostic ability since few positive slides are encountered. To meet this need we will provide sets of diagnostic slides. The material will be gynecological. The slides will be sent out as unknowns and need not be returned. Two days later, a summary of the clinical findings and cytologic diagnosis will be sent.

Those interested should write to Dr. Ruth M. Graham, Roswell Park Memorial Institute, Buffalo, 3, New York, U. S. A.

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AUGUST 31 to SEPTEMBER 2, 1961
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INVITATION TO ATTEND AND PRELIMINARY INFORMATION

In the past two decades a new discipline has come to be of major importance in the diagnosis of early cancer—exfoliative cytology. It has been well established that cancer can be diagnosed earlier by means of exfoliative cytology than by any other means at present. Exfoliative cytology has also proved useful in studying the behavior of early lesions, and is becoming of increasing importance in the assessment of hormonal status.

To acquaint cytologists, clinicians, and pathologists with the new developments in this rapidly expanding field, an International Congress of Exfoliative Cytology is being held in Vienna, Austria, from August 31 to September 2, 1961. All interested in the field of exfoliative cytology are invited to attend.

Since the International Congress of Exfoliative Cytology will be immediately followed by the Third World Gynecology Congress, the main emphasis of this meeting will be on the exfoliative cytology of gynecology and obstetrics, though the exfoliative cytology of other specialties will not be excluded from the program.

The main topics of the Congress are:

1. Progression, Persistence, and Regression of Epithelial Anomalies (Reversibility and Irreversibility of Carcinoma in Situ)
2. Hormonal Activity as Reflected in the Cytological Specimen in Patients with Gynecologic Tumors or Breast Cancer.

In addition to these two themes of the Congress there will be general papers on various topics of exfoliative cytology and cytological research.

Simultaneous translation in four languages will be provided (English, French, German, and Spanish).

The complete transactions of the Congress will be published in ACTA CYTOLOGICA, the Journal of Exfoliative Cytology. Scientific medical exhibits are planned for the Congress.

The Congress Bureau has chartered an airliner from an international scheduled airline for persons (including their families) from the Americas planning to attend the Congress. This plane is scheduled to leave New York on August 19 (to Paris or Frankfurt) and scheduled to leave (for New York) on September 12. The price for the round trip fare is less than $280.00.

IF YOU ARE INTERESTED IN ATTENDING OR PARTICIPATING IN THE INTERNATIONAL CONGRESS OF EXFOLIATIVE CYTOLOGY, AND/OR WISH TO HAVE MORE INFORMATION, PLEASE RETURN THE ATTACHED REPLY CARD *AS SOON AS POSSIBLE.*

USE THIS HANDY REPLY CARD TO ANSWER AS SOON AS POSSIBLE

International Congress of Exfoliative Cytology
666 Elm Street, Buffalo 3, New York, U.S.A.

Gentlemen:

I am interested in attending or participating in the International Congress in Vienna, Austria (Aug. 31–Sept. 2, 1961), and wish to have more information about the meeting. Please add my name to your mailing list:

Name: (Please print)_____

Street:_____

City:_____

Country:_____

I am interested in the reduced fare flight, New York–Paris (or Frankfurt)–New York ☐ Yes ☐ No (Check or circle answer which applies, please)

I am interested in attending the Third World Gynecology Congress (Sept. 3–10, 1961) after the International Congress of Exfoliative Cytology ☐ Yes ☐ No

I am interested in receiving information about subscription to ACTA CYTOLOGICA, the Journal of Exfoliative Cytology ☐ Yes ☐ No

LETTERS TO THE EDITORS

ADMINISTRATION OF ESTROGENS AS PROGNOSTIC TECHNIQUE DURING PREGNANCY

TO THE EDITORS:

In 1953 van Meensel, Lambert and I (1) showed that the administration of 100 mg dienestrol in threatened abortion accompanied by proliferative cytological changes caused increased changes in the abnormal cytology, if the placenta was profoundly altered and the expulsion of the degenerated products of pregnancy imminent.

Since then we have not seen any exception to this rule, and we use this technique in order to establish the poor prognosis of pregnancy once the uterus decreases in size and does not correspond any longer to the age of pregnancy. The ideal dosage is, conforming with the results of Pundel and von Meensel, 100 mg dienestrol orally for three days.

It may happen under these conditions that in the beginning of a threatened abortion the cytological pattern exhibits even more the pronounced characteristics of the pregnancy pattern, but this reponse is transitional and the reaction becomes typical of an estrogenic pattern. It is likely that the placenta shows a tendency to return to normal, but the genetic, circulatory or inflammatory conditions make such a recovery impossible. As a matter of fact, histologically in these cases the placenta is always rather strongly altered. When after the sixth week the cytological pattern of the pregnancy appears more highly estrogenic in type, we administer 50 mg dienestrol per day for ten to twenty days, and we observe concurrently a quick and lasting accentuation of the cytological characteristics peculiar to normal pregnancy.

On the other hand, the administration of 125 mg 17-hydroxyprogesterone caproate every other day and simultaneously 50 to 100 mg dienestrol orally every day for eight to ten days does not inhibit the typical estrogenic reactions of the vaginal epithelium if the pregnancy is already definitely disturbed. The facts stated do not yet have a valid explanation, because the hormonal interrelations during pregnancy still have too many knknowns. This is not only a protective mechanism of the pregnancy and of the fetus against high amounts of estrogen. Why does the organism destroy with such an ease the various and multiple estrogenic substances administered to date? We are not faced with an inertia of the vaginal epithelium, since we, like other authors, were able to currently observe positive estrogenic reactions, perhaps, however, somewhat diminished for the two estrogens introduced directly in the vagina: diethyl-stilbestrol and ethinyl-estradiol either during or after pregnancy, in the case of persistence of the atrophic vaginal epithelium.

There is without a doubt a luteotrophic reaction caused by these substances with estrogenic activity. This is known under the name of Hohlweg reaction, and it is present in the experimental animal only when the pituitary gland is intact. The latter uses estrogen to secrete a "prolactin-like" substance. Thus, when estrogens act as a favorable influence on the pregnancy, the signs of mammary hypertrophy increase. The dark pigmentation of the areoles after administration of diethylstilbestrol is different and points out, however, that this substance is not immediately destroyed after its administration.

We have undertaken numerous studies on rats concerning the increase of prolactin secretion under the influence of massive dosages of estradiol benzoate. It is possible that the pituitary gland during pregnancy only responds to sufficient amounts and that the status of pregnancy modifies in this sense the reactions of the pituitary to estrogens. The increase of luteotrophic hormone has as a consequence increased secretion of progesterone, wherever it may be produced: in the placenta, in the adrenals or in the corpus luteum of pregnancy. The latter could possibly undergo a functional reactivation at this time; perhaps atretic but functional corpus lutea could grow under these conditions.

Our hypothesis is in agreement with that of Pundel and Horalek and Sonek. The prolongation of this treatment might lead to a regression of the luteotrophic reactions and thus explains the statements of Gaudefroy. The regressing pregnancy might render the pituitary

more or less suddenly in a refractory state. Our hypothesis has the advantage of a solid experimental basis.

The direct examination in isotonic solution under the phase contrast microscope allows an easy and quick control of the cytological reactions under hormonal treatment. We now use only this method and we take as the criteria the proliferative features of the cells and the Karyopyknotic Index.

We also put great emphasis on the degree of the flatness and size of the desquamated superficial cells. Our findings are recorded in a cytogram that has a foundation in these various cytological patterns.

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ADVANTAGES OF THE USE OF THREE-DIMENSIONAL CONDENSER FOR HORMONAL CYTOLOGY

TO THE EDITORS:

The aim of colpocytological examinations is partly to screen out carcinoma cases and partly to establish the hormonal diagnosis.

In our institution, in Budapest, Hungary, in order to establish the diagnosis rapidly, a fresh, unfixed specimen is examined under a special microscope. With this method staining becomes superfluous and the preparation is immediately ready for examination. Unstained preparations also can be examined with a regular bright field microscope with the Abbe's condenser lowered and the diaphragm narrowed. However, the image thus obtained does not show the outlines of vaginal epithelial cells, nor the nuclear structure with sufficient clarity, and the mobile morphological elements are seen by their motion rather than by their form. The native microscopic image produced by lowering the Abbe's condenser has the further disadvantage of being difficult to use for photomicrography because of the resultant poor illumination.

The introduction of the phase contrast microscope meant a great advance in the study of fresh preparations. If the preparation is sufficiently thin, the cellular borders are sharply defined and the nuclear structure can be well visualized. Thus, diagnosis of karyopyknosis is easy and also some cytoplasmic details may be strikingly visualized. Using the phase contrast microscope on fresh specimens, the color differences rendered by Papanicolaou's staining method cannot be visualized, but, on the other hand, the advantages concomitant with the quick evaluation of fresh, unfixed preparations have been gained.

Contrary to the above condenser systems which work in two dimensions, Professors Koss and Laszlo Zselyonka have constructed a condenser which enables an estimation of the relative cellular thickness in the microscopic picture, i.e., it produces a three-dimensional picture.

The main feature of this condenser lies in the fact that only a small part of the light beams passing through it reach the condenser unchanged. An extremely thin caesium mirror covers the condenser so that only a narrow band is left open in the center. The light intensity is thus reduced to 20%. To eliminate part of the light-beams, an opaque disc is placed in the side of the condenser facing the objective. Using the auxillary tube the refractive system is adjusted in such a way that the greyish caesium mirror and the opaque blocking disc are focused and the lower pole of the disc is placed at the border of the visual field (Fig. 1). At the upper part of the visual field, light beams of Type I enter the microscopic lens system unchanged, with 100% intensity and 550 $M\mu$ wave length. This assures the unilateral oblique illumination of the specimen. The light beams of Type II retain only 20% of their intensity and their wave length is shorter than that of the former type. These beams illuminate the circular side. Type III light beams show the blocking disc in the position where the light intensity is zero. Type IV light beams come from the invisible part of the condenser to the object under examination and originate from the lower part of the light band between the caesium mirrors. (The auxillary tubes do not show this light band.) The Type IV beams are oblique ones with a dark

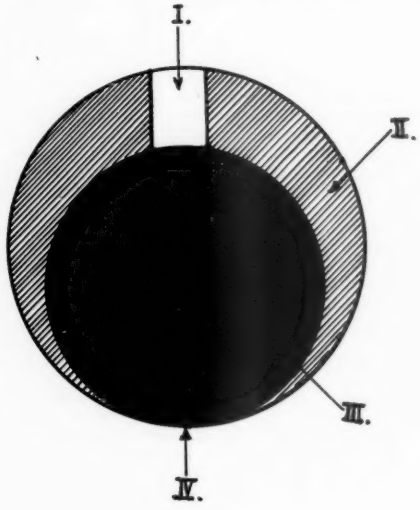

Fig. 1.

visual field. With such an arrangement of light, Koss and Zselyonka succeded in turning the specimens to achieve a shadow effect which results in a plastic appearance of the specimen.

It must be noted that a similar picture based on oblique illumination was also produced at the end of the last century. It was done partly by shifting the condenser and partly by unilateral covering of the mirror. More recently the cellular appearances produced by Michel, Geller and Reincke are, depending on the light refraction, more or less distorted and do not possess the advantage of the double increase in resolving power, which is an important feature of the three dimensional condenser.

When preparing the specimens, we use the following technique: a drop of fluid is taken with a glass rod from the blade of the posterior vaginal speculum and mixed with one drop of NaCl solution. This is spread carefully and covered with a cover glass. It is important that only a small amount of vaginal fluid be mixed with the NaCl solution, because the vaginal epithelial cells should be in a monocellular layer. Quick drying of this prepared specimen may be avoided for days if it is placed into a moist chamber after framing the cover glass with melted paraffin.

Using this new condenser, the fresh specimens can be seen in three dimensions. This is an entirely new microscopic appearance, because, instead of the formerly obtainable two-dimensional images, the cells are seen in depth. Their thickness can be accurately determined, and the site of their nuclei and the cytoplasmic granulations also are seen.

Examining the specimens at a 200x magnification, the vaginal microbiology can be easily classified. With their protuberant semispherical shape, leukocytes differ strikingly from the epithelial cells. They have already been shown to appear flatter under the oil immersion objective. Their shape is round and the nuclei protrude only slightly from the level of the cytoplasm and the coarse nuclear chromatin granulations, as well as the fine cytoplasmatic granulations which can be distinctly visualized. Trichomonas vaginalis is easily recognized by its shape and its vigorous movements. Its flagella are also seen in three dimensions. The constant waving of the undulating membrane is easily noted. The plastic appearance does not show the nucleus of the Trichomonads. Trichomonads respond with great sensitivity to differences in osmotic pressure. In a hypotonic NcCl solution they become markedly swollen within one to two minutes, and it is only at this time that they are as large as usually observed in the dried vaginal smear. In this form they are already significantly larger than the leukocytes.

The fungoid filaments of spores occurring in the vaginal discharge likewise appear in three dimensions.

The three dimensional condenser was used also for examining sperm in the course of infertility studies. The head and neck of the sperm are seen in three dimensions. Due to the protrusion of the cellular nucleus the apex of the head, representing about 1/3 of it, appears to be flat.

When discussing the cellular elements of the vaginal fluid, the cells belonging to the surface and those to the deeper layers must be dealt with separately. In the three dimensional condenser the appearance of the superficial layer of the vaginal epithelium is characterized by the pyknotic nucleus which shows definite structure and emerges very distinctly from the smooth surface of the polygonal cell. The cytoplasmic granules protrude from the cells. The cellular boundaries and surfaces, respectively, distinctly show traces of connections with neighboring cells, which under the phase contrast microscope are to be seen only in the form of some irregularities.

The large polygonal cells with non-pyknotic nuclei, differ mainly in their nuclear structure from the above cells. The nucleus emerges very slightly from the cell; its structure is striking and a circular granulation is seen at the nuclear margin. The cytoplasmic granulation is similar to that of cells with pyknotic nuclei.

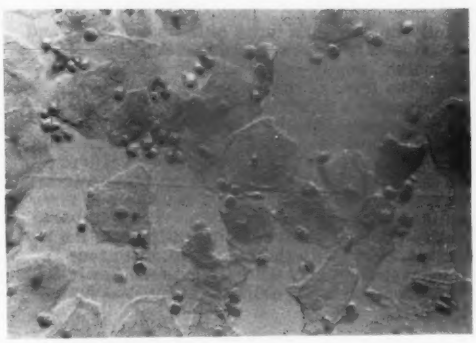

Fig. 2. Mainly superficial epithelial cells. Colpitis. A great number of leukocytes.

Fig. 3. Secretory phase. The cells are predominantly navicular vaginal epithelial cells.

Fig. 4. Colpitis vetularum. The difference in thickness between the superficial and parabasal cells is very distinct.

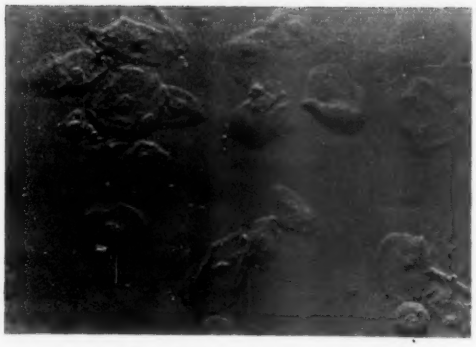

Fig. 5. Hypohormonal cytological appearance. The cells originating in the deeper layer of the vaginal epithelium predominate.

The cells of the parabasal layer appear in an entirely new form on the shadowed microscopic image. These cells are significantly thicker than those of the superficial and intermediate layers. The nuclei of these roundish, singly-lying cells do not emerge from the level of the cytoplasm; moreover, in some cases a small retraction corresponding to the nucleus may be observed. The granulation of both the cytoplasm and the nucleus is slightly coarser. As a rule, these cells are so thick that, using the phase contrast microscope, the cellular boundaries cannot be visualized with sufficient clarity. The parabasal cells, protrude characteristically from their base. Thus, with their definite difference in thickness they may be distinguished easily from the other epithelial cells of the vaginal epithelium.

The colpocytological findings can be easily evaluated in the fresh specimen with the aid of their appearance. Due to the peculiar light refraction the vaginal epithelial cells and the other formed elements of the vaginal fluid can be seen in a shadowed manner. In addition to their shape and clustering, the thickness of the cells also helps in the establishment of an accurate diagnosis. As can be seen, the deeper the vaginal epithelial layer from which the cells originate, the thicker they are. To recognize karyopyknosis with this method is easier than with any other procedure described up to the present, for the pyknotic nuclei are three dimensional and emerge like a ball from the surface of the superficial epithelial cells.

As compared with the phase contrast method the advantage of the three dimensional procedure is the fact that vital staining can also be applied when examining the fresh specimens, and thus their evaluation becomes even easier. Because of its cheapness, the three dimensional microtechnical process described here enables everyone to examine native colpocytological preparations, and the establishment of a quick hormonal diagnosis is rendered possible.

The three dimensional condenser as a method of cytological examination on fresh specimens gives an entirely new microscopic appearance. The vaginal epithelial cells originating from the different layers are recognized without difficulty, partly on the basis of karyopyknosis and partly by recognition of their cellular thickness. With this method we can accurately classify the microbiological content of the vaginal fluid. Due to its simple construction, extended applicability and cheapness, the three dimensional image is held to be very practicable in performing hormonal colpocytologic studies.

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THE SYMPOSIA BY CORRESPONDENCE OF ACTA CYTOLOGICA

INTRODUCTORY REMARKS

The Symposia of ACTA CYTOLOGICA are *held entirely by correspondence* and contain international discussions of scientific problems of interest to the exfoliative cytologist.

System for Selecting Subjects for Symposia: From recommendations received, the Editorial Office will draw up the list of subjects and will publish these subjects in ACTA CYTOLOGICA, under the heading FUTURE SYMPOSIA.

Instructions for Authors: Each problem will be introduced by a *Main Speaker* or *Speakers*. These principal papers will then be considered by persons identified as *Discussants*. As a general rule, approximately 600 words each will be allocated for main papers and 200 words each will be allocated for the contributions of the Discussants. The Main Speakers will then be given the opportunity to make unlimited Closing Remarks.

Photomicrographs and tables may be reproduced: one full page for each principal paper and for the paper of the Discussant (maximum one-half page per contribution). The photomicrographs and tables should be submitted in glossy photographic prints, preferably in the size of 4 × 5 inches (i.e., 10 × 12 cm) and should show a proportional 10μ scale on its reverse side. *Each figure should be accompanied by a comprehensive caption.*

The Discussants are requested *to strictly restrict their contributions to the discussion of the main papers.* Discussions which are not directly related to the main paper cannot be accepted. It is suggested that the Discussants prepare their contributions in such a manner that the reader may gain the impression of an actual round table conference.

The Closing Remarks of the Main Speakers should be limited to the answering of questions raised in the discussion and to other directly related information.

The Bibliography for the papers of both Main Speaker and Discussant should be organized in the same manner as in the American Journal of Obstetrics and Gynecology, at the *end* of the paper. *Every cited opinion or publication should have a reference in the bibliography.*

Deadline for Contributions: The Editorial Office will set deadlines for each written symposium. These will include:

1. deadline for agreements to contribute.
2. deadline for main papers.
3. deadline for discussions.
4. deadline for closing remarks.

Reprints: Authors may receive reprints of their papers by ordering these reprints before the particular issue goes to press. There will be a nominal charge for reprints: $6.00 per page for the first one hundred copies, and $3.00 per page for each additional hundred.

LES SYMPOSIA PAR CORRESPONDANCE DES ACTA CYTOLOGICA

Les *Symposia par Correspondance* des ACTA CYTOLOGICA présentent des discussions internationales sur des problèmes scientifiques intéressant le cytologiste exfoliative.

Système du choix des sujets pour les symposia: En partant des propositions, et sous la rubrique: FUTURS SYMPOSIA, le bureau de rédaction dressera la liste des sujets principaux qui seront publiés dans les ACTA CYTOLOGICA.

Recommandations pour les auteurs: Chaque sujet principal sera présenté par un Rapporteur Général ou des Rapporteurs. Ces mémoires principaux seront alors soumis aux Participants à la Discussion. En règle générale 600 mots seront accordés aux Rapporteurs des sujets principaux, et, 200 mots aux Participants à la Discussion. Les Rapporteurs Généraux pourront clôturer les discussions par un nombre illimité de remarques.

Des microphotos et graphiques pourront être reproduits à raison d'une page entière pour chaque sujet principal et une demi page au maximum pour les discussions. Les microphotos et les graphiques doivent être présentés sur du papier brillant, de préference dans le format 10×12 cm. *Chaque figure devra être accompagnée d'une legende explicative précise.*

Les membres et invités prenant part aux discussions sont invités à *limiter strictement leurs interventions aux discussions des sujets principaux.* Des discussions qui n'ont pas de rapport direct avec le sujet principal *ne pourront être acceptées.* Il est recommandé que les discussions soient rédigées d'une manière telle que le lecteure ait l'impression d'assister à une discution réelle de table ronde.

Les Remarques de Clôture du Rapporteur Général devront se limiter à la réponse aux questions soulevées dans les discussions et aux autres informations éventuelles ayant un rapport direct avec le sujet.

La bibliographie des rapports et discussions devra être rédigée de la même manière que celle de l'American Journal of Obstetrics & Gynecology et figurer à *la fin* du texte. *Chaque opinion ou publication citée dans le texte doit avoir sa réference dans la bibliographie.*

Dates limite pour les collaborations: Le bureau de rédaction, fixera des dates limites comprenaet:

1. un délai pour l'acceptation des collaborations,
2. un délai pour les sujets principaux,
3. un délai pour les discussions,
4. un délai pour les remarques de clôture.

Tirés-à-part: les auteurs pourront obtenir des tirés-à-part de leurs communications en les demandant avant la mise sous presse des ACTA CYTOLOGICA publiant leurs articles. Les tirés-à-part seront facturés: $6.00 par page de texte pour le premier cent et $3.00 pour chaque centaine supplémentaire.

DIE SCHRIFTLICHEN SYMPOSIEN DER ACTA CYTOLOGICA

Die schriftlichen Symposien der ACTA CYTOLOGICA befassen sich auf internationaler Basis mit wissenschaftlichen Problemen, die für den Exfoliativ-Zytologen von Interesse sind.

System der Thema-Auswahl für die Symposien: Die Schriftleitung stellt auf Grund von Thema-Vorschlägen eine Liste von Haupt-Themen zusammen, und gibt diese Liste unter dem Titel ZUKÜNFTIGE SYMPOSIEN bekannt.

Instruktionen fur Autoren: Jedes Thema wird von einem oder mehreren Referenten behandelt. Diese Referate werden dann von Diskussions-Vortragenden besprochen. Im allgemeinen werden Referate auf etwa 600 Worte beschränkt, und Diskussions-Vorträge auf 200 Worte. Die Referenten erhalten dann die Gelegenheit, Schlussbemerkungen ohne Wortzahlbeschränkung zu machen.

Mikrophotographien und Tabellen können abgedruckt werden: eine Ganzseite kann Referenten und eine halbe Seite Diskussionsvortragenden für Abbildungen zur Verfügung gestellt werden. Die Photographien sind auf Hochglanzpapier, und möglichst in der Grösse 10 × 12 cm erbeten und soll ein proportionales 10μ Zeichen auf der Rückseite haben. *Jede Abbildung muss von einem erklärenden Untertitel begleitet sein.*

Die Diskussionsvortragenden sind gebeten, sich in ihren Beiträgen *streng an das Hauptreferat zu halten.* Diskussionsbeiträge, die sich nicht an das Hauptthema halten, *können nicht berücksichtigt werden.* Es wird vorgeschlagen, dass die Diskussionsvorträge in einem Stil abgefasst sind, dass der Leser den Eindruck gewinnt, als ob es sich um eine Diskussion am runden Tisch gehandelt hätte.

Die Schlussbemerkungen der Referenten sollen sich nach Möglichkeit auf die Beantwortung von Diskussionsfragen beschränken.

Die Bibliographie der Referate und der Diskussions-Vortäge sollen *am Schluss* der Beiträge nach dem Muster der Bibliographien im American Journal of Obstetrics and Gynecology aufgeführt werden. *Jede zitierte Ansicht oder Publikation muss eine Referenz in der Bibliographie haben.*

Termine für Beiträge: Die Schriftleitung setzt Termine für die Schriftlichen Symposien fest. Die folgenden Termine werden bekanntgegeben:

1. Termin für Erhalt der Beitrags-Zusagen,
2. Termin für Erhalt der Hauptreferate,
3. Termin für Erhalt der Diskussions-beiträge.
4. Termin für Erhalt der Schlussbemerkungen.

Sonderdrucke: Autoren können Sonderdrucke ihrer Beiträge bestellen, bevor die betreffende Ausgabe in Druck geht. Die Schriftleitung muss diese Sonderdrucke berechnen und wird einen Betrag von $6.00 pro Seite und 100 Sonderdrucke, und einen Betrag von $3.00 für jedes weitere Hundert erheben müssen.

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El simposium escrito de ACTA CYTOLOGICA contiene discusiones internacionales sobre problemas científicos que son de interés para el citólogo exfoliativo.

Sistema de selección de materias para el simposium: Con sugestiones recibidas, la oficina editorial confeccionará una lista de los temas más interesantes, lista que será publicada en ACTA CYTOLOGICA con dos numeros de anticipación a la fecha de su posible publicación, bajo el epígrafe de "SIMPOSIUM FUTUROS."

Participación en el Simposium Escrito: No habrá restricción alguna sobre el número de puntos de discusión en los que cualquier autor desee participar.

Instrucciones a los Autores: Cada problema deberá ser presentado por un ponente o ponentes. Estos trabajos principales serán entonces discutidos por los comunicantes. Como regla general, se permite un máximo de 600 palabras para los trabajos principales y 200 palabras para las contribuciones de los comunicantes. Al ponente principal se le da la oportunidad de hacer rectificaciones finales ilimitadas.

Pueden reproducirse microfotografías y tablas: una página por cada trabajo principal y un máximo de media página por discusion. Las microfotografías y tablas deberán enviarse en forma de copias fotográficas amplias. A ser posible de 4 × 5 pulgadas (10 × 12 cms). *Cada figura deberá acompañarse de su correspondiente leyenda.*

Se suplica a los comunicantes *ajustar estrictamente sus comunicaciones a la discusión de los trabajos principales.* Las discusiones que no estén directamente relacionadas con el trabajo principal *no podrán ser aceptadas.* Se sugiere que los comunicantes realicen sus contribuciones de manera tal que el lector tenga la impresión de estar ante una verdadera mesa redonda.

Las rectificaciones finales de los ponentes deberán limitarse a contestar las preguntas aparecidas a lo largo de la discusión asi como a otras directamente relacionadas con el tema.

La bibliografia, tanto de las ponencias como de las comunicaciones deberá redactarse de la misma forma que figura en el American Journal of Obstetrics and Gynecology, *al final* del trabajo. *Toda opinión o publicación citada deberá tener su correspondiente referencia en la bibliografía.*

Fechas para las contribuciones: La Oficina Editorial, fijará fechas límite absolutas para cada simposium escrito. Estas incluirán:

1°. Fecha límite para acuerdo de contribución,
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Symposium

EFFECTS OF ENDOGENOUS ESTROGENS ON THE VAGINAL EPITHELIUM

INTRODUCTION: SOURCES OF ENDOGENOUS ESTROGENS

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Estrogens are not only formed in the ovary but also in the placenta, testicle and the adrenals are sources of these bodies. Possibly they may also originate, although in minimal quantities, in some other tissues. There are places of physiological formation, but under certain conditions tumors or hyperplasias of certain organs become sources of estrogens and constitute sites of abnormal production: a study which is of great interest in understanding the biogenesis of these steroids (7, 8).

I. The Ovary

A. Physiologically: Studies with radioactive phosphorous, after the injection of FSH (1) have demonstrated that the ovary not only forms estrogens in the granulosa and in the theca of the mature follicle, but also in the fibrous thecal masses, in the atretic follicle, in the cortical theca and in the theca of the corpus luteum. In this manner we get the impression (2) that it is thecal tissue that is the more active and important producer of estrogen.

B. Pathologically: It is well known that there is formation of estrogens in tumors of granulosa and theca: as a classical statement, it has been accepted that every woman with postmenopausal estrogenic activity has a tumor of this type, even if only microscopically. However, a closer examination of the ovaries of women with continued excretion of estrogens after menopause has revealed that, in the absence of a functional tumor, hyperplasia of the cortical stroma (12, 15) will secrete estrogens in sufficient quantity to maintain a proliferative phase in the vagina or the endometrium or to stimulate a growth of certain malignant tumors (4).

II. The Placenta.

Historically it is known that there is formation of estrogen in chorionic tissue (2). Recent evidence of placental estrones has been obtained by direct extraction (6), by the culture of placental tissue (5), and by perfusion with G14 precursors (10). The

neoplastic formation of chorionic tissue, mole, and chorioepithelioma do not secrete estrogens in appreciable quantities. This can be explained because these formations reproduce immature chorionic tissue and estrogenic production is a property of mature chorionic tissue.

III. The Adrenals.

A. Physiologically: The adrenal production of estrogens has been assumed in physiological conditions during embryonic life (2) and after the menopause. Estrogenic production is stimulated by ACTH (14) and can be obtained in profusion from adrenals with or without this treatment. (9, 19). We admit today that the adrenal cortex as an organ is capable of secreting, in any moment, supplemental estrogens.

B. Pathologically: In certain varieties of rats and mice, castration produces an adrenal hyperplastic reaction with hyperproduction of estrogens (2). Certain forms of adrenal hyperplasia in humans are feminizing (17, 21). It is possible to admit that in adrenal hyperplasia exercise may produce a stimulating action against certain carcinomas of the breast and uterus (4, 11). The same applies to suprarenal tumors.

IV. The Testicles.

A. Physiologically: The production of estrogens produced in the testicles is universally admitted (20). The source of origin has been thought to be Sertoli's cells, but it is possible that Leydig's cells may accomplish this mission, since in tumors of the latter cells gynecomastia sometimes appear (20).

B. Pathologically: There are some feminizing tumors of the testicle, the most vivid example being the so-called feminizing testicle (3). They are masculine pseudohermaphrodites, with a total feminine bearing and with well-developed breasts. Castration produces atrophy of the breast and the remaining feminine characteristics. Feminizing testicular formation has been described by us (3) as an adenoma (a constant finding) in all cases.

V. Other Tissues?

Estrogens are biosynthesized by a relatively simple mechanism starting from the polymerization of acetate(8, 9, 10). This synthesis possibly can be carried out in some other tissues, (today unknown), probably in the liver. The resection of both ovaries and adrenals does not suppress estrogenic excretion in breast cancers (4, 11, 19). That can only totally disappear with hypophysectomy (11). The latter is a last endogenous source of estrogens obviously existing, but still obscure.

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DISCUSSION

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Dr. Botella Llusia has written an excellent summary of the sources of endogenous estrogen. There are two items in his presentation with which I am not in full accord: I know of no direct proof of estrogen secretion by ovaries showing cortical stromal hyperplasia; hypophysectomy does not necessarily eliminate estrogen secretion.

To discuss the latter point first, in Currie's book (2), Bulbrook (p. 181-186) and Scowen (p. 208-213) report that some patients with breast cancer continue to excrete urinary estrogens after hypophysectomy. Subsequent reports have been made (1, 4). Since then, other laboratories have confirmed these findings. Therefore, there seems to be general agreement that hypophysectomy does not necessarily eliminate urinary estrogen excretion.

Ovarian cortical stromal hyperplasia is a tantalizing subject. I have observed isolated instances of patients with postmenopausal vaginal bleeding, finally terminating in total hysterectomy with bilateral salpingo-oophorectomy. Typical "swiss

cheese" endometrial hyperplasia and cortical stromal hyperplasia with thecosis were the only significant abnormalities found on pathological examination.

Smith et al. (6) first described this entity and its possible relationship to endometrial hyperplasia and carcinoma. More recently Sommers et al. have stressed the relationship between ovarian cortical stromal hyperplasia and breast cancer, describing a much higher incidence of such ovarian architecture in patients with breast cancer than in a control group. These authors are emphatic that women with advanced breast cancer whose ovaries (removed at castration) showed cortical stromal hyperplasia had a longer survival than those whose ovaries were atrophic.

Our studies in women with advanced breast cancer have been unable to confirm this observation. There seemed to be no relationship between ovarian morphology and response to therapeutic castration or duration of life following castration. Furthermore, there was no relationship between ovarian morphology and pre-operative vaginal smear studies.

In summary, while a relationship of cortical stromal hyperplasia to certain neoplasms has been postulated, and there is suggestive evidence that cortical stromal hyperplasia may be associated with endocrine activity, there has been no direct proof of estrogenic secretion by such ovaries.

These comments should not be interpreted as detracting from Botella Llusia's concise and excellent presentation.

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CLOSING REMARKS

JOSÉ BOTELLA LLUSIA:

Finkbeiner's observations are reasonable. The effect of hypophysectomy on the estrogenic secretion is still not clear in carcinoma of the breast. While Bulbrook and Scowen believe in continuance of estrogenic excretion after hypophysectomy, Luft, Olivecrona and other (page 27 of quoted book) have observed improvement of carcinoma of the breast after hypophysectomy. As improvement is not always possible, estrogenuria apparently does not always disappear following hypophysectomy. We should not forget that part of the urinary estrogenic excretion in women derives from food or from direct synthesis of acetic groups, which can take place in organs other than in the endocrine glands. On the other hand, not all the Kober positive chromogens similar to those found by Bulbrook and Scowen are estrogenic as Dicfalusy and others have been able to demonstrate (1). With regard to the ovarian cortical stromal hyperplasia, I mentioned it because I do not want to contradict those who believe in it while I do not have any evidence of the opposite. We only have doubts concerning its estrogenic productive capacity (2). Instead, we believe in the important role of the adrenal cortex in the paradoxical formation of estrogens in senile women. It seems, nevertheless, that both hypotheses raise objections and since we do not know how estrogen is formed in humans and animals, there remain many questions which will be left unanswered.

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THE RELATIONSHIP BETWEEN THE APPEARANCE OF VAGINAL SMEARS AND THE RATE OF EXCRETION OF URINARY STEROIDS

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There is little information available on the relationship existing between the appearance of vaginal smears and the endocrine activity of the ovaries and of the adrenal cortex. A possible method which may be employed to study this problem is to study the relationship between the vaginal smears and the quantity of the different steroids present in the urine.

Before presenting these results we shall give in the first part of this work an account of statistical work which has been carried out on the appearance of vaginal smears in ovulatory cycles as well as in the different categories of amenorrhea. It is important to know, if, by using any one given method for sampling, staining and reading vaginal smears, it is possible to give a cyto-vaginal diagnosis of insufficient estrogenic effect, or of hyperestrogenic effect or, between these two conditions, of the normal estrogenic impregnation of the vaginal mucosa at any chosen period of the menstrual cycle. The diagnostic value of such a clinical method of investigation is dependent on the existence of a clearly defined normal range for the results it can provide.

I. MATERIAL AND METHOD

Cytological study of vaginal smears

A. Technique

The sampling is carried out by the patient herself by means of a swab. The smears are spread on a glass slide, one end of which is frosted where the name and date are noted. The smears are dried in air and placed in a ridged box which prevents their rubbing against each other.

The slides are arranged in series of 20 at a time and are passed through the following baths:

1. distilled water - 5 minutes (hydration)
2. Mayer's acid Haemalum - 10 minutes.
3. slightly alkaline water - 10 minutes
4. the cytoplasmic stain - 10 minutes

Reagent:		
	Orange G . . .	0.15 g.
	Eosin	0.30 g.
	Light green . .	0.075 g.
	"Bleu lumiere"	0.45 g.
	Phosphomolybdic acid .	0.20 g.
	Phosphotungstic acid . . .	0.15 g.
	Acetic acid . .	2 ml

Complete with ethyl alcohol 50% to 100 ml.

5. ethyl alcohol baths of increasing concentration: 80-85-90-95-100% (dehydration).
6. two toluene baths (clearing agent)
7. mounted in Canadian balsam

The slides are then read by means of a Reichert fibroscope with a special fitting for projection onto a ground glass screen at 170x and 500 x magnifications.

B. Criteria for interpreting results

The differentiation of the cells is based solely on the size and structure of the nucleus. Thus, in principle, the haemalum-eosin stain could suffice for the cytological diagnosis; but, in actual fact, we prefer to use a differential stain which stains the cytoplasm pink and blue-green. By this means it is easier to distinguish the outline of the nucleus which facilitates the readings and makes for greater precision.

We distinguish four main categories of cells:

1. cells having a pyknotic nucleus, i.e., having a small, rounded and hyperchromatic nucleus (with a diameter of less than 7), and having an eosinophilic cytoplasm;

2. cyanophilic cells with a large nucleus: a well defined nuclear membrane, the nuclear substance is clear and contains a nucleolus and several clumps of chromatin material (the diameter is greater than 10);

3. cells having a small nucleus, intermediate in appearance between the well-defined nucleus of the preceding category and that of the pyknotic nucleus in the strict sense of the word. The nucleolus has disappeared and the structure of the nucleus appears denser than the cyanophilic cells just described (the diameter is between 7 and 10), and the cytoplasm can be either eosinophilic or cyanophilic;

4. parabasal cells of the deeper layers.

The nuclear structure provides criteria for classification which are just as important as the actual size of the nucleus itself. The other characteristics of vaginal smears are secondary but are nevertheless noted: i.e., whether the cells are isolated or in clumps, and the presence of leukocytes, mucus, and microbial or parasitic flora.

As a result of preliminary statistical research work and also of our own clinical experience, it appears that the percentage of karyopyknotic cells varies in parallel with the percentage of cells having a small nucleus. This preliminary work deals with two hundred different menstrual cycles chosen at random. Figure 1 shows this conclusion and demonstrates the parallel variations of the percentage of karyopyknotic cells and small nucleated cells as a function of the large nucleated cells. For this reason we have included the sum of the karyopyknotic and small cells under the denomination of "small nucleated cells (S. N.C.)." We have abandoned distinguishing between eosinophilic and cyanophilic cells; although we have used this method over a considerable period of time using Shorr's reagent and fixing the smears in alcohol-ether. In our hands this distinction is not a sufficiently well-defined and reliable criterion and is subject to many sources of error.

At least six to eight vaginal smears per cycle were taken for all the menstrual cycles studied. These vaginal smears were divided into three categories: smears of the ovulatory phase (OS), smears of the luteal phase (LS corresponding to the premenstrual thermic plateau and smears of the follicular phase (FS), taken between the end of the menstrual period and the sixth and eighth day before the temperature change. For the follicular and luteal phases the results are expressed in averages.

C. Estimation of steroids

The estimation of the phenolsteroids (PS) was carried out using the method of Jayle et al. (1), after hydrolysing the conjugates with enzymes of Helix Pomatia's digestive juice (600 units of glycuronidase and about 300 units of sulphatase per ml of urine.). These phenolsteroids are expressed as microgram equivalents of estrone using a special modification of the Kober reaction. The pregnandiol (PG) was estimated by the method of Crepy, et al. (2), after enzymatic hydrolysis. The 17-ketosteroids (17-KS) were estimated using the Zimmermann method according to the modified technique of Jayle et al. (3).

II. RESULTS

The smears have been divided into the three periods as mentioned above: follicular, ovulatory and luteal.

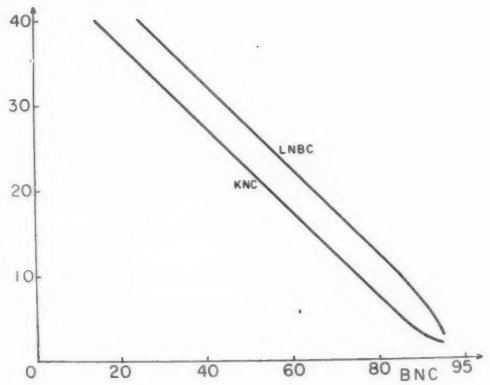

Fig. 1. The variation of the karyopyknotic nucleated cells (K.N.C.) and the small nucleated cyanophilic cells (S.N.C.).

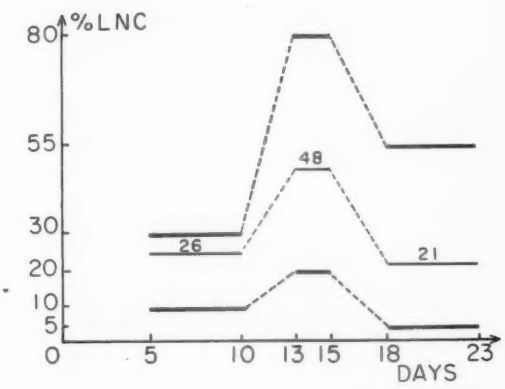

Fig. 2. The solid lines represent the average percentage of the SNC in the three phases studied (follicular, ovulatory and luteal); the dotted lines correspond to the phase not studied.

TABLE I

Average and limiting values of the normal of the percentage of S.N.C. in the follicular, ovulatory and luteal phases of 439 menstrual cycles

	S.N.C.		
	Average percentage	σ	Limiting values for 95% of the cases
Follicular phase	26	8	10 to 30
Ovulatory phase	48	18	20 to 85
Luteal phase	21	11	5 to 55

TABLE II

Average and limiting values of the percentage of S.N.C. in 164 cases of amenorrhea

	Number of cases	Average percentage	σ	Limiting values for 95% of the cases
Amenorrheas with FSH> 50 m.u.	67	7.5	3.5	0 to 15
Amenorrheas of more than two years duration with FSH> 5<25	97	9	6	0 to 25

Menstrual cycles

Vaginal smears were studied in 439 cases of women between 20 and 40 years of age having approximately normal cycles with a thermic plateau of 8 to 12 days, normal excretion of 17-KS (5 to 13 mg/24 hrs. by our method) and normal pregnandiol values (4 to 10 mg/24 hrs. by our method).

Table I shows the results obtained in this group and in Figure 2 the limits are shown of the percentages of SNC during the follicular, ovulatory and luteal phases. It is thus possible to outline areas of normal and hypoestrogenic effect on the vagina during the follicular and ovulatory periods of the menstrual cycle.

We consider that the vaginal smears during the menstrual cycle show hypoestrogenic effect when all or nearly all of the readings are situated between the axis of the abscissa and the lower limit of the normal zone. (Fig. 2). In fact, hypoestrogenic effect on the vagina can be appreciated only by studying all of the smears taken together as a whole. The definition of hypoestrogenic effect on the vagina as studied during the study of ovulatory menstrual cycles is corroborated by comparing them with smears taken from women with different types of amenorrhea.

Amenorrheas

This work deals with a group of 164 women with amenorrhea, which have been subdivided into two categories according to whether the amount of urinary gonadotropin was high (FSH> 50 m.u.) or normal (FSH> 5<25). Table II gives the average results expressed as percentages of SNC.

In the first group* (with elevated FSH) the percentages of SNC in two-thirds of the cases were lower than 10% and lower than 15% in 95% of the cases. The mean results and distribution of the SNC were about the same in the second group with normal FSH levels.

* This group is called the "hypergonadotropic amenorrheas."

III. THE RELATIONSHIP BETWEEN THE PERCENTAGE OF SNC AND THE RATE OF EXCRETION OF URINARY STEROIDAL METABOLITES

Phenolsteroids and Small Nucleated Cells

A. We have studied the correlation between the relative number of SNC and the amount of phenolsteroids excreted in normal follicular and ovulatory phases, as well as in 122 women with amenorrhea. Bravais and Pearson's correlation coefficient is 0.58. It is highly significant at the threshold P<0.01. The average value of SNC was taken for each increment of phenolsteroids of 5μg between 0 and 60 μg. The results are indicated in Figure 3.

TABLE III

The levels of phenolsteroids in μg./24 hrs. and percentages of S.N.C. during amenorrhea, in the three three different phases of the menstrual cycle and during early pregnancy.

		Hypergonadotropic amenorrhea	Follicular phase	Ovulatory phase	Luteal phase	Early pregnancy (5-6 wks)
PHENOLSTEROIDS μg./24 hrs.	Mean values	10	17	46	27	115
	σ	5	8		9	
	Limiting values for 95% of the cases	0 to 20	5 to 30		15 to 55	
S.N.C.	Average percentage	7.5	17	48	19	22
	Limiting values for 95% of the cases	0 to 15	10 to 30	20 to 85	5 to 55	
Number of cases		67	105	15	614	32

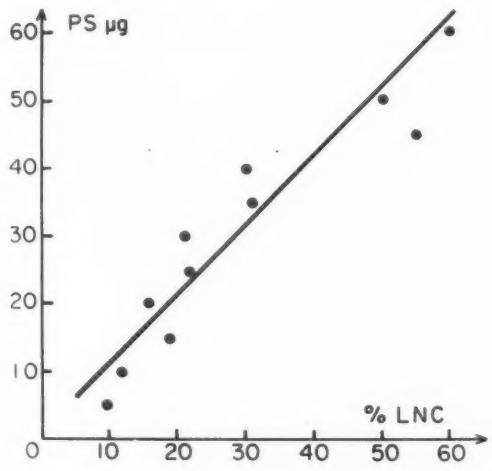

Fig. 3. The correlation between the number of little nucleated cells (SNC) and the total phenolsteroid excretion (PS).

B. In 68 patients of the above group from whom vaginal smears were obtained during the follicular and ovulatory periods, the correlation between the percentage of the SNC in the ovulatory and follicular phases and the amount of phenolsteroids excreted between the 7th and 9th day of the cycle was determined. A correlation coefficient $r = 0.28$ significant at the threshold $P < 0.02$ was found.

C. Considering the results shown in Table III and in Figure 3, it is apparent that there is some relationship between the mean phenolsteroid level and the percentage of SNC in hypergonadotropic amenorrheas and the follicular and ovulatory phases of the menstrual cycle.

In a previous study (4) we found a mean phenolsteroid level of 8.6μg (σ=6) in 47 cases with amenorrhea and a mean level of 16μg (σ=9) in 84 normal follicular phases. The standard deviation of the differences of the averages being 1.3, we deduced that the difference in phenolsteroid levels between the two categories was greater than 4μg which is confirmed by the results obtained in Table III. In other words, the ovary secretes an appreciable quantity of estrogen in the post menstrual period which on an average is twice that found in women with amenorrhea.

TABLE IV

The relationship between the phenolsteroid level (PS) in the luteal phase and the percentage of S.N.C. observed in the follicular, ovulatory and luteal phases of the menstrual cycle.

Excretion of PS in the luteal phase µg./24 hrs.	Percentage of S.N.C.		
	Follicular phase	Ovulatory phase	Luteal phase
<10	13	31	15
11-15	21	41	16
16-20	22	44	17
21-25	20	43	17
26-40	26	52	18
> 40	22	57	28

TABLE V

The relationship between the relative number of S.N.C. values in the follicular and ovulatory phases and the phenolsteroid values in the luteal phase according to whether the 17 ketosteroid rates are low, normal or high.

	17 ketosteroids in mg/24 hrs.		
	< 6.5 mg (88 cases)	6.5 to 13.5 mg (439 cases)	> 13.5 mg (87 cases)
Follicular phase	r= 0.03 nil	r= 0.22 highly significant at P 0.01	r= 0.43 highly significant at P 0.01
Ovulatory phase	r= 0.09 nil	r= 0.26 highly significant at P< 0.01	r= 0.34 highly significant at P< 0.01

In the luteal phase the phenolsteroid level increases as compared to that of the follicular phase, while the percentage of SNC is about the same as in the follicular phase. Thus, it follows that there is no longer a correlation between the number of SNC and the phenolsteroid levels, as was the case in the amenorrhea group and in the preluteal period of the menstrual cycle. This lack of correlation between the phenolsteroid levels and the relative number of SNC is even more obvious in early pregnancy. Even when the phenolsteroid level rises to four times the value found in the luteal phase, the SNC values remain practically identical (Table IV). This shows that under the influence of progesterone secretion the vaginal mucosa no longer reacts to the keratinizing effect of the estrogens.

D. The correlation between the percentages of SNC at different phases of the menstrual cycle and the rate of excretion of the phenolsteroids in the luteal phase was studied (see Table IV).

In our routine method for the functional investigation of the corpus luteum the urine is collected on the fifth day of the premenstrual thermic plateau in order to carry out the test in the middle of the luteal phase. This explains why we have far more phenolsteroid evaluations in this phase of the cycle than in the other periods of the cycle.

As it is presumed that estrogens are formed in the thecal cells of the corpus luteum, we wondered whether or not there was a relationship between this estrogenic activity of the corpus luteum and the relative number of SNC during the ovulatory and follicular phases. Table IV supplies an affirmative answer to this question.

In Table V our results are classified into three categories according to whether the 17 KS levels were low, normal or elevated. The correlation indices are calculated in each category both in the follicular and ovulatory phases.

1. Follicular phase

The correlation coefficient, r 0.03, is nil for the first group of 88 cases with low 17-KS excretion. On the other hand it is 0.22 for a group of 437 cases with normal elimination of 17-KS and 0.43 for a group of 87 cases with high elimination of 17-KS. In the last two

TABLE VI

The relationship between the relative number of S.N.C. and the phenolsteroids levels as a function of the urinary pregnandiol levels.

	Pregnandiol in mg/24 hrs.	
	< 5.5 mg (227 cases)	> 5.5 mg (387 cases)
Luteal phase	r= 0.09 nil	r= 0.25 highly significant at P<0.01

TABLE VII

The relationship between the relative number of S.N.C. and the urinary pregnandiol levels of the luteal phase, according to whether the 17-ketosteroid rate is low, normal or high.

17-KS rates mg/24 hrs.	Number of cases		Averages	σ	r
< 6.5	88	% S.N.C.	19	12	-0.05
		PG mg/24 hrs.	5	1.9	nil
6.5 to 13.5	439	% S. N.C.	21	11	0.09
		PG mg/24 hrs.	6.7	2.4	nil
>13.5	87	% S.N.C.	17	10	0.19
		PG mg/24 hrs.	8.3	3.6	slightly significant at P< 0.05

groups, although this correlation coefficient is low, it is highly significant at the threshold P< 0.01.

2. Ovulatory phase

The results obtained by comparing the percentage of SNC in the ovulatory phase with the phenolsteroid levels of the luteal phase were found to be parallel with the results in the follicular phase for these three categories. The correlation coefficient is nil (r=0.09) in the first group and becomes highly significant, although still low, in the other two groups: r= 0.26 and 0.34.

E. As was seen earlier, if the results obtained in the amenorrheas and normal menstrual cycles are pooled, the correlations which exist between the SNC and the phenolsteroids are to be found in phases other than the luteal phase. Nevertheless, this does not mean that there is no relationship between the phenolsteroid levels and the SNC values during the luteal phase. To study this aspect our cases were divided into two categories according to whether the urinary pregnandiol levels were 5.5 mg or lower (0.5 to 5.5 mg) or higher (5.6 to 13 mg) per 24 hrs (Table VI).

In the group with low or subnormal pregnandiol the correlation coefficient between the percentage of SNC and the phenolsteroid level was nil (r = 0.09). But on the contrary, when the pregnandiol level was higher than 5.5 mg, the correlation coefficient was found to be 0.25 which, although still low, is significant at a threshold of P< 0.01. Hence, there is a slight correlation between the percentage of SNC and the phenolsteroid level only in the case of sufficient progesterone production.

Pregnandiol and the Small Nucleated Cells (S.N.C.)

Table VII gives the results found in the three groups having low, normal and high 17-KS levels. No correlation could be found between the PG level and the percentage of SNC in the different groups. It should be noted that the mean PG values were found to be higher in the third group than in the other two, which can be explained by the fact that the adrenals excrete pregnandiol precursors in cases where there is hyperactivity of the androgenic function of the adrenal cortex.

We have tried to ascertain in another experiment whether there is a significant differ-

TABLE VIII

The relationship between the relative number of S.N.C. and the urinary 17-ketosteroids during the menstrual cycle according to whether the 17-ketosteroid levels happen to be low, normal or high.

17-KS rates in mg/24 hrs.	Percentage of S.N.C.		
	Follicular phase	Ovulatory phase	Luteal phase
< 6.5	25	45	19
6.5 to 13.5	26	48	21
>13.5	22	47	17

ence in the number of SNC in the ovulatory and luteal phases or, in other words, between the pregnandiol level and the decrease in SNC between ovulation and the luteal phase. For this purpose 196 luteal phases having a normal phenolsteroid level of between 25 and 40μg were studied. The correlation index, r = 0.24, is significant at the threshold P< 0.01. From this one can deduce that there is some relationship between the progesterone activity of the corpus luteum and the decrease in SNC in the luteal phase.

17-Ketosteroids and Small Nucleated Cells (S.N.C.)

Table VIII indicates the average percentages of SNC in the follicular, ovulatory and luteal phases according to whether or not the rate of excretion of 17-KS was low, normal or high.

A statistical study of these values confirms the impression gained from this Table in which it appears that there is no correlation between the rate of excretion of the 17-KS and the percentage of SNC. This correlation coefficient when calculated for the ovulatory phase is r 0.05, indicating complete absence of correlation between the 17KS level and the appearance of the vaginal smears.

IV. DISCUSSION

From our personal experience the number of SNC represents the best cyto-vaginal test for the production of estrogens. In the first part of this work limits of normal for small nucleated cells (SNC) were able to be defined during different phases of the menstrual cycle. The establishment of such normal zones during the cycle seems to us extremely important as it enables one to define the zone of hypoestrogenic activity. This is also confirmed by the distribution of SNC observed in amenorrheas. It may be concluded that vaginal smears furnish valuable information of great diagnostic value for women who still menstruate and whose number of SNC falls in the area outlined by the axis of the abscissa of the lower limit of the normal zone. If, instead of accepting the rather broad interpretation of the criteria for the clinical normality of our cases, we had had at our disposition a sufficiently large group of normal women, then the normal area of the average curve would probably have been somewhat reduced on all sides of the average SNC curve (Fig. 1).

We found that, on the whole, there was a satisfactory statistical correlation between the amenorrheas and during the follicular and ovulatory phases of the menstrual cycle. This confirms the observations made by Young et al. (5) and Puttarajurs et al. (6). The former found that a correlation of r= 0.33 was significant at the threshold P= 0.02 in women who were menopausal or who had been castrated. The latter found statistical correlation between the sum of the three estrogens as estimated by Brown's method and the percentage of karyopyknotic cells of the vaginal smears of the second day after collecting the urine from the follicular and ovulatory period; but on the other hand this correlation was not found during the luteal phases of the four different cycles studied.

In our opinion, three main categories of vaginal smears can be taken into account during the first ten days of the menstrual cycle:

1. the first category, normally estrogenic, includes those having SNC percentages between 10 and 30%.

2. the second category, which is hypoestrogenic, with SNC values of 0 to 10%.

3. the third category, which is hyperestrogenic, with SNC values of 30 to 80%.

On the whole, these limits correspond to excretion of phenolsteroids of from 15 to 30μg in the first category, from 0 to 15μg in the second, and from 30 to 80μg in the last category. Using this technique it is striking to note that the phenolsteroid values observed when expressed as μg/24 hrs. are comparable with the number of SNC expressed as percentages (Fig. 3). The mean phenolsteroid level is found to be 10μg and the SNC count 7.5% in hypergonadotropic amenorrhea; in the follicular phase of the menstrual cycle the mean level of the phenolsteroids was 17μg while in the same group of subjects the SNC counts were 17%; and at the time of ovulation the average percentage of SNC is 45% and the Phenolsteroid level 50μg.

As this just happens to be a fortuitous statistical coincidence, one should not try to deduce the quantity of phenolsteroids excreted per 24 hours from the percentage of SNC present during the first part of the menstrual cycle. It is nevertheless true that if one uses the size of the nucleus as a means of differentiating the cells, the vaginal smears provide clinical proof, which is of definite diagnostic value, of estrogenic activity by the ovaries. The cycles that have been studied may be divided into three categories according to whether the SNC curve is high, in the normal area, or situated in the area of hypoestrogenic activity.

We believe that the discouragement of a large number of authors, whose opinions on the value of vaginal smears has been all too pessimistic, is based on the fact that they used the eosinophilic reaction as the criterion of estrogenic impregnation. When using the size of the nucleus as criterion, it cannot be doubted that the symptomatological value of vaginal smears with low SNC values is greater than that of smears with high SNC values. In other words, the diagnosis of hypoestrogenic activity is more valid than that of hyperestrogenic activity. During the luteal phase of the menstrual cycle or in early pregnancy the vaginal mucosa becomes insensitive to the action of estrogens of both endogenous and exogenous origins. Also no differences were found between the percentages of SNC despite the fact that the phenolsteroid rates were four times higher during early pregnancy than during the menstrual cycle. This observation apparently disagrees with the fact that there was definite correlation, though small, between the percentages of SNC in the luteal phase and the phenolsteroid rates in the same period of the cycle. In order to explain this relationship two factors must be taken into account: (1) The relative decrease of SNC from the time of ovulation to the luteal phase is about the same from one cycle to another when the ovulation peak is sufficiently high and when the progesterone production is sufficient. This explains why the higher the ovulation peak the higher the SNC count will be in the luteal phase. (2) The second factor is to point out the relationship between estrogen production by the corpus luteum in the luteal phase and the estrogen activity of the ovary during the follicular and the ovulatory phases. The same endocrine tissue, that is to say, the theca interna of the follicle, produces estrogens at the time of ovulation and during the period of luteal production; and also as we have already shown, there is a weak but significant correlation between the SNC percentages in the follicular, ovulatory and luteal phases, and the phenolsteroids of the luteal phase. It is possible to understand through these explanations that there is a certain relationship, though slight, between the SNC counts and the phenolsteroid levels, even though the vaginal mucosa has become insensitive to the action of the estrogens. When the estrogen activity is normal, one finds a slight but significant correlation between the SNC counts and the pregnandiol excretion in the luteal phase. However, above a certain threshold of progesterone secretion no difference is found at all, and this explains why there is no difference between the SNC in the middle of the luteal phase and in early pregnancy.

One must not deduce from this set of facts that there do not happen to be certain individual facts that are contradictory. We encountered a certain number of cases showing a state of virilism, probably of ovarian origin, in whom we observed a discrepancy between the hypoestrogenic activity of the vagina and appreciable quantities of urinary phenolsteroids. It appears that abnormal androgen production by the ovary is often associated with an excretion of phenolsteroids. We even believe that this discrepancy between the hypoestrogenic appearance of the vaginal smears and the level of urinary estrogens is a sign of ovarian virilism.

Another surprise arising from our statistical work is the lack of correlation between the 17-KS level and the appearance of the vaginal smears. In fact, it is classically considered that the urinary 17-KS reflect the androgen activity in the urine, and in women the activity of the androgen function of the adrenal cortex. One could be led to suppose that for a given quantity of estrogen the percentage of SNC would be inversely proportional to the 17-KS rates. However, this was not so, because in cases with relatively high levels of 17-KS, the appearance of the vaginal smears were quite normal when the menstrual period was normal. One must deduce, therefore, that the adrenal precursors of the 17-KS have no anti-estrogenic action on the vaginal mucosa although it has been well established that these androgens may have a virilizing effect. This does not signify that the adrenal androgens have no effect at all on the vaginal mucosa. Although in the cycles with depressed 17-KS levels no correlation was found between the phenolsteroid rates in the luteal phase and the estrogenic impregnation of the vagina in the first part of the cycle, such a correlation does exist when the 17-KS rates are either normal or high. We cannot explain this phenomenon except that it may be possible that the adrenal androgens have some effect on the receptivity of the vaginal mucosa to estrogens. Hence, it would appear that the vaginal smears lose their symptomatological significance when the adrenal production of androgens is insufficient. It may be concluded from the above that virilism has an effect on the appearance of the vaginal smears which is very different according to whether the virilism is of ovarian or adrenal cortical origin. In the first case the vaginal smears have a hypoestrogenic aspect, whereas in the second they are not influenced at all as long as regular menstrual cycles persist. Indeed, it is a classical fact that in congenital hyperplasia of the adrenals the menstrual cycle is blocked by the androgenic hyperactivity of the adrenal and the vaginal smears become sub-atrophic. This atrophy is not caused by the action of androgens on the vagina but the alteration of the gonadotropic function of the pituitary body which causes a cessation of the menstrual cycle and, consequently, the atrophic condition of the vaginal mucosa.

SUMMARY

The number of SNC (karyopyknotic + small nucleated cells) constitutes a good indication of the estrogenic activity of the ovaries in the follicular and the ovulatory phases, as witnessed by the significant correlation obtained with the urinary phenolsteroid levels.

In the luteal phase the vaginal mucosa no longer reacts to estrogenic stimulation but the SNC level in this phase depends, to a certain measure, on the relative number of SNC found

in the previous follicular and ovulatory phases. There is also a correlation between the phenol-steroid rates on the fifth day of the premenstrual thermic plateau and the relative number of SNC in the previous follicular and ovulatory phases. In addition, a slight correlation was found between the pregnandiol rate and the decrease of SNC in the luteal phase.

The relative number of SNC in the different phases of the cycle is independent of the 17-ketosteroid rates.

A statistical study of 439 ovulatory cycles and 164 amenorrheas made it possible to outline the normal averages and limits of SNC values in different phases of the cycle. A hypoestrogenic zone of the vaginal smear has thus been defined.

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DISCUSSION

JOHN A. FINKBEINER, New York, New York, U.S.A.:

Dr. Jayle and his co-workers have presented some very interesting data in an extremely difficult field. In our present state of ignorance, it is not possible to measure the actual amounts of hormones secreted by the various endocrine glands. Such measurements must depend on indirect methods; in this presentation, exfoliative vaginal cytology and the urine excretion of certain steroids. Before the results of the comparison of these two different methods can be appreciated, it is necessary to understand exactly what is being measured, what is comparable.

Urinary steroid assay measures the concentration of various hormone metabolites appearing in the urine. The accurate quantitation of these metabolites does not necessarily reflect their physiologic activity in the body, for the exact relationships between hormone secretion, action, metabolism, utilization, and excretion has not been established. Therefore it seems rather remarkable that there has been such good correlation between urine estrogens and vaginal smears.

To the best of my understanding, Jayle's method of estrogen assay is fairly similar to that of Brown (1) with the exception that the chromatographic separation into estrone, estriol, and estradiol is not done. Is it fair to assume that the "phenol steroids" are comparable to the "total estrogens" of Brown, the numerical sum of the amounts of estrone, estriol and estradiol found to be present? Are the same objections against Brown's method valid against Jayle's, namely are some estrogenic substances (2, 3, 4) not detected by his method?

The measurement of 17-ketosteroids is not a precise measurements of androgen secretion; those androgens which do not have the 17-keto configuration are not included in the results and conversely, not all steroids having a 17-keto group are androgens.

Vaginal smears reflect endocrine activity by alteration in proliferation, differentiation, and desquamation of the vaginal cells. While it is true that only estrogen will cause significant differentiation in the cells of the vaginal mucosa, I am not convinced that it is reasonable to attribute all degrees of differentiation solely to proportionate amounts of estrogenic substances, totally ignoring the effect of other hormones.

Dr. Pearson, his co-workers, and I have been engaged in similar studies in which the urinary estrogens have been measured by the Brown method and by bioassay, and the vaginal smears interpreted by the differential cell count technique as outlined later in this symposium. Most of our patients were women with advanced breast cancer and treated by oophorectomy adrenalectomy and hypophysectomy singly or sequentially. The urinary estrogens ranged from 0 to 40 gamma daily, with the majority of the determinations in the lower ranges. Generally speaking, there was good correlation between the results of the Brown method, the bioassay, and the vaginal smears with the range above 15 - 20 gamma of estrogens/24 hours. However, with the lower values, particularly below 10 gamms/24 hours, the correlation became progressively less - sometimes there was good correlation between the two of the three results, but the three methods were seldom in complete agreement. Furthermore single determinations were generally less precise than serial determinations.

These results are not unexpected, for it is generally agreed that the accuracy of either the Brown method or the bioassay decreases as the lower levels are approached. In this range of estrogen level, the vaginal smear may vary from a completely atrophic smear to a proliferative pattern, that is, a nonspecific type of pattern.

From the differential cell count technique of vaginal interpretatíon, the "vaginal smear index" as well as the "eosiniphilic index" and the "karyopyknotic index" may be calculated. The data indicated that the vaginal smear index has better agreement with the urine assay. While the correlation of all three was satisfactory with the higher levels of estrogenic activity, the vaginal smear index was equally satisfactory in the higher levels but was far superior in the lower levels. This result could be anticipated since only the "vaginal smear index" expressed the total vaginal smear pattern.

The term "SNC" confuses me, for despite the definitions, I have the impression the "SNC" is different than "karyopyknotic index." Perhaps Jayle will clarify this point.

As Jayle has noted there is consid-

erable "overlap" in the vaginal smear activity in various patients, in various phases of the menstrual cycle, and with various menstrual or endocrine histories. This is clearly shown in the Tables by the relative magnitude of This has certainly been my experience. Furthermore, since hormones other than estrogen can effect the vaginal smear, I have found it extremely hazardous to predict a definite level of estrogenic excretion (particularly with the lower levels of endocrine activity) such as Jayle does in Figure 3.

While unquestionably such a statistical correlation can be made, its value in a single isolated determination is questionable. Undoubtedly serial determinations, wither urinary assay or vaginal smears, are more valuable than an isolated observation.

In summary, there is a correlation between vaginal smears and endocrine activity. With our present inadequate understanding of indocrine function and our limitations in testing their metabolic activities, this correlation is surprisingly good.

Congratulations to Jayle and co-workers on their detailed study.

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NO CLOSING REMARKS

HISTOLOGICAL MODIFICATIONS OF VAGINAL AND CERVICAL EPITHELIUM AND CONNECTIVE TISSUE DUE TO PHYSIOLOGICAL PRESENCE, DEFICIENCY OR ABSENCE OF ESTROGEN STIMULATION

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The study of the effects of castration was greatly modified in 1923, when ALLEN and DOISSY (1) by injection of estrogenic substances, induced keratinization of the vaginal epithelium. This process permitted the quantitative measurement of the estrogenic action of the liquor folliculi. Since that time, it has been possible to establish the logarithmic law of the action of the morphogenetic hormones (2, 3, 4, 5) and demonstrate the effects of grafts and injection of estrogens into castrated subjects (6, 7). However, the study of hormonal equilibrium and, consequently, the interpretation of histological images of the vagina and cervix, are still difficult in the case of menopausal women because of certain adrenal secretions and, in particular, the increased level of the 17-ketosteroids (8).

Nevertheless, it has been possible to study the effects of the isolated action of the estrogens on the vagina and the cervix in the rat and in certain women ovariectomized and adrenalectomized for breast cancer.

Three elements seem necessary in order to understand the elective action of the estrogens on the tissues of the genital system:

I. The embryological unity of the entire female genital tract, which is derived from the Muller's duct.

II. The action of the estrogens on the metabolism of the connective tissue.

III. The elective action of the estrogens on complete maturation with cornification and pyknosis of the epithelial cells.

I. Embryological unity of the female genital tract

From Müller's duct are developed the oviducts, the uterus, the cervix and the upper third of the vagina. But Müller's duct itself is simply a derivative of the Wolffian duct, as was verified by Nagel in 1889, and as Peter Gruenwald (10) seems to have demonstrated in 1941 and 1943, on chick and human embryos.

In fact, Müller's duct originates from the cleavage of the Wolffian duct, and Müller's cells are derived from the Wolffian cells. The Wolffian duct itself arises from an invagination formed in the nephrogenetic tissue produced by the coelomic mesoderm. This origin would account for the multipotentiality of the constituants and their specific receptivity to the steroid hormones, but does not really explain their extraordinary sensitivity. Nevertheless, this explanation, though incomplete, facilitates an understanding of the coordinated way in which the various constituents of the genital tract respond to hormonal stimuli; whether they be of connective, muscular or epithelial character, they react according to their differentiation. And a reflection of their common origin is sometimes found in tumor pathology (botryoid sarcoma, carcino-sarcoma, so-called mixed tumors of the uterus, clear-cell tumors, etc.).

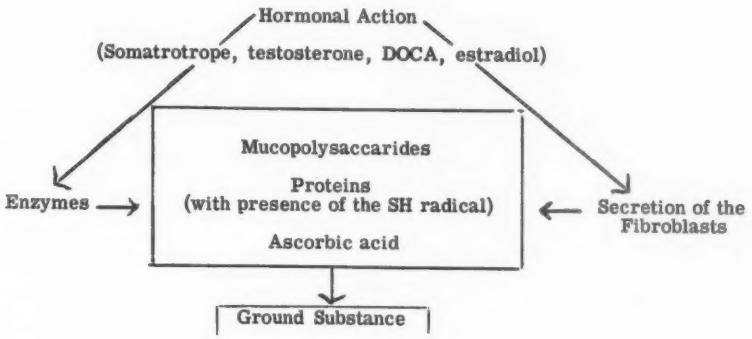
II. Action of the estrogens on connective tissue

Since 1950, when Klemperer (11) enunciated the concept of "collagen disease," attention has been directed to the ground substance modifications produced by the action of the hormones. We shall not discuss here the resultant theories of "polymerization and de-polymerization" of the glycoproteins under the influence of the steroids, as they are beyond the scope of this paper. In reality, three different hormonal effects should be distinguished, namely, those on:

A. The ground substance itself,
B. The fibrillogenesis, and
C. The cells.

A. Action on the ground substance

Sub-microscopically, the ground substance is formed of mucopolysaccharides in combination with proteins at their iso-electric point, with more or less marked degree of polymerization. In other words, the consistency of the ground substance depends on the state of its molecular organization. The following diagram assumes approximately the conditions of its formation:



This ground substance is in a constant state of alteration, owing to the enzymatic and vascular phenomena governing the homeostasis, as was shown by Joseph Engel and H. Catchpole (12). By electrochemical methods these authors studied the concentration of the negatively-charged immobile colloids. From the thermodynamic point the colloids are organized as a double-phase system:

- a phase of high-concentration colloid, low in water-content, and
- a phase of low-concentration colloid, high in water-content.

The two phases are in reciprocal equilibrium.

By the freeze-drying technique, Bondareff has shown that under the electronic microscope the ground substance appears as a vacuolar system in which the vacuolar content, of low density, is surrounded by a continuous zone of higher density.

Very recently Dennis (14) has shown the distribution of grains of ferrocyanide to be (not in the blank spaces, but in the ground substance itself) in the form of infinitesimal droplets, 600 to 1,200 A in diameter. A distribution of this ferric salt in the interior of the vacuoles may be represented as the test of the high water-content phase of the ground substance, since modifications in the density of the distribution of the ferrocyanide imply a change in the ionic equilibrium of the ground substance. Such modification in ferrocyanide distribution occur in relation to the hormones injected, which change the two-phase colloidal equilibrium by varying proportions, by modifying the hydration.

According to the now-classic conclusions of Gersh and Catchpole (15), an increase in the solubility of the PAS staining components of the ground substance partially reflects its degree of hydration. This notion is important for an understanding of the action of the estrogens on the ground substance itself. Electrometric studies, confirming the conclusions of Gersh and Catchpole, have shown that hormones decrease the total calcium-magnesium concentration and the ionic concentration of sodium and potassium.

Khron and Zuckerman (16), later Thorn and Engel (17), and finally Zuckerman, Palmer and Hanson (18) studied water metabolism as influenced by the genital hormones. They found that the injection of estrogens determines an increase in the water content. As a result, the ground substance swells, disintegrates and stains more readily with PAS.

B. Action on fibrillogenesis

In a relatively recent study we, with Du Boistesselin (19) investigated the fibrillogenesis on the mesentery in rats treated with different hormones. This study was made directly, with the phase-contrast microscope, on the exposed mesentery of the anesthetized animal. Whereas in the control animal the fibers appeared long, straight, enmeshed and of nearly equal thickness, those in the mesentery of the estradiol-treated animals were very slender, hardly visible, and especially much less numerous than in the control. The ground substance was much looser, more fluid and abundant. It should be noted that the action of cortisone greatly exaggerated this aspect, whereas the somatotrophic hormone, the DOCA and testosterone increased especially the number, thickness and undulant figures of the fibers. The reticulin was likewise modified in comparison to the control and was much less distinct, less well-formed.

C. Action on the cells

The fibrocytic elements composing the stroma are modified by estrogenic action, and there are noted:

a hypertrophy and a hyperplasia,
a multiplication of the elements,
a distinct tendency to angiogenesis.

By means of histological examination of the inflammatory granuloma in the rabbit or

the rat, numerous authors have been able to study the action of the hormones on connective tissue cells. In particular, Taubenhaus and Amromin (20) emphasized the stimulative action of the estrogens on the growth of granulation tissue. Whereas this is true for malnutritioned or ovariectomized animals, in our experiments (21) on non-castrated animals subjected to high doses of estrogens we found that the connective tissue cells were nearly normal in number, but smaller in size than in the control animals, and their cytoplasmic expansion less marked, but their capacity to form capillaries, on the other hand, was the same as, or even greater than, in the controls.

These observations - opposed to those of Taubenhaus and Amromin - in reality confirm them. For, whereas in the castrated animal the estrogens have in fact a stimulative action on the cells, in high doses their action on the cells and the ground substance is different and recalls somewhat that obtained by cortisone (reduction and size of the fibroblasts, edema of the ground substance).

The suppression of estrogens determines, by modifying the ionic equilibrium, a high-concentration colloid phase, low in water-content, of the ground substance, which then appears denser, and only faintly PAS staining or negative. The fibrillogenesis disappears. The cellular elements are rare and small, and angiogenesis ceases. Once the estrogens are introduced into the circulation, all these factors tend to return to normal.

III. Action of the estrogens on the cells of the malpighian epithelium

The estrogens have a two-fold action on the malpighian epithelium covering the ectocervix:

a) growth stimulation of the germinative layer by cellular multiplication, and
b) determination of complete cellular maturation.

Although all the genital hormones have a good trophic action on the malpighian epithelium, only the estrogens are capable of effecting its complete maturation. However, it should be added that the estrogens may, in certain cases, exceed the normal limits, whereas this is not the case with the other genital steroids.

These latter, and in particular testosterone and the progestational substances are moderately stimulant to the growth of the germinal cells, even when given in large or even excessive quantities.

The estrogens, by contrast, intensely accelerate the growth and multiplication of the germinal cells and, as we shall see under another topic, activate the reserve columnar cells and thus induce epidermoid metaplasia. The suppression or large deficit of estrogens causes a rapid disappearance of the eosinophilic cells, the pyknotic cells, and then of the intermediate cells of the middle layers C_2 and C_4; finally, the only cells remaining stationary are those of the internal basal layer, often reduced to a single layer of cells. It then easily follows that this exulceration of the epithelium becomes an ulceration bleeding on contact, and readily infected. But these inflammatory phenomena are always very superficial, as if the density of the subjacent connective tissue prevented the diffusion of the infection. Furthermore, it suffices to give, over a period of four or five days, a few gammas of estrogens for the disappearance of this inflammation and the reappearance of the mucosa, which rapidly will turn brown on the Schiller test.

The functional activity of the ectocervical epithelium is evidenced by the glycogen, which appears only if there exists an exchange between the cervical stroma and the epithelium. An absence of estrogens prevents the formation of this glycogen.

The biochemists believe that the accumulation of glycogen occurs in connection with the diminution of oxygen. Dempsey and Wislocki (22) have even established a close relationship between the glycogen deposit and the activity of the aerobic oxidation. Meyer and MacShane (23), in turn, have shown the inhibitory effect of the estrogens on the oxidizing enzymes. This might then explain the presence of glycogen in the cellular layers C_3 and C_4.

The estrogens are the only steroids capable of provoking complete cellular maturation: they induce pyknosis, eosinophilia, and sometimes even abnormal keratinization, probably by action on the S.H. protein metabolism, which enters into the constitution of keratohyalin. However, the mechanism of this action is not well known.

We have seen that a good trophic state of the connective tissue is essential for the maintenance of the malpighian epithelium in good condition. But the question could be raised, why the exchanges between epithelium and connective tissue do not likewise occur in the direction: epithelium to connective tissue. We can answer this question only by tissular comparisons. Dunnington and Smalter (24) in a very precise study of the relationship between the cornea and its epithelium, have shown that, in the connective tissue of the cornea, the synthesis of some ground substances is effectuated by the conjugate and perhaps synergestic action of the covering epithelium and the connective tissue itself. The epithelial cells are probably the source of the energy, and the necessary precursors to the synthesis of the chondro-sulfate and the kerato-sulfate.

Donn and Maurice (25) have studied the movements of sodium between the connective tissue and the corneal epithelium and have observed a movement of sodium transportation from the epithelium toward the connective tissue. Furthermore, the experiments of Virginia Weimar (26) have shown that the presence of the epithelium is necessary for the development of fibroblasts in the connective tissue, as the epithelium is the source of a proteolytic enzyme having a factor activating the stroma cells.

All these facts, as yet little known, open new perspectives concerning the intimate physico - chemical action of the estrogens on the tissues, not only on the microscopic, but also on the sub-microscopic and biochemical scale.

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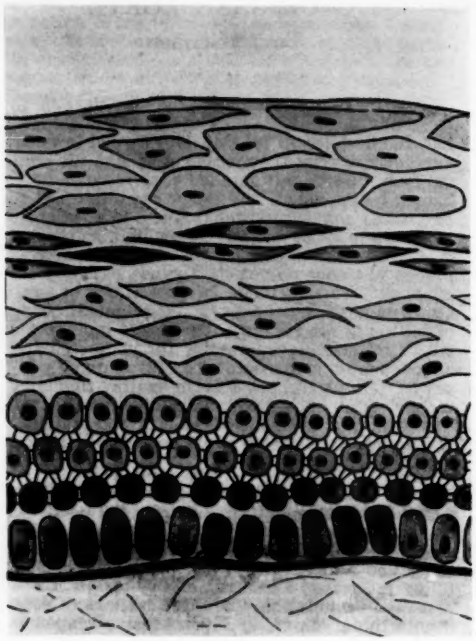
In establishing "type" pictures of fixed histological changes one has to consider that the histologist is generally forced to relate excised material from various women for comparison. Using this method possible errors cannot be excluded. As every organism has its individual character, conclusions in such comparisons must be drawn cautiously. Furthermore, one has to demand that the examined pieces of tissue do not show gross pathological changes. This includes inflammations of any kind, neoplastic processes and chemical changes of milieu of the vagina. The tissue picture must not be injured mechanically, for instance by descensus or prolapse of the uterus or by pessary. One should not deform healthy tissue by biopsy. One should adopt a middle course, which makes obtaining tissue necessary and simultaneously accomplishes a great deal of the mentioned demands.

The known histological picture of the vaginal membrane at the height of the proliferative phase represents the cardinal point of consideration for estimation of the absence, deficiency and physiological presence of estrogens. In the following, I understand by endogenous estrogen stimulation, the effectiveness of estradiol in the organism and the reactive response of the mentioned tissues to this substance. The semischematic sketch of the "cornified" type by Papanicolaou and Traut (see Fig. 1) will serve as basis for the discussed conceptions. This sketch has been slightly altered. The listed histological terms correspond to the opinion of Sobotta. The epithelium of the portio vaginalis uteri, (ectocervix) according to principle, responds to all hormonal influences just as does the vaginal epithelium. Differences in the gauge of the layers of both regions are in the main explained by different vascularization. The "richer culture medium" produces sappy epithelium. The portio, the subepithelial vascular connective tissue of which tends to hyaline fibrosis after abortion or post partum, has naturally an epithelial layer which is measurably thinner. In the vaginal epithelium, cornification (as in the skin) occurs only because of mechanical duration, irritation or desiccation; both, for instance, found with prolapse.



Stratum corneum

C_5 Stratum lucidum

C_4 Stratum granulosum

C_3 Stratum superficiale

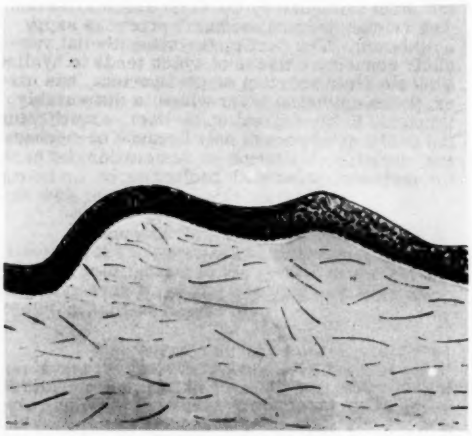
Stratum spinosum

C_2 Stratum profundum

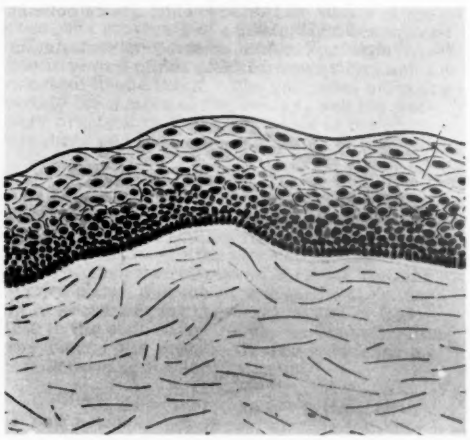
C_1 Stratum cylindricum

Fig. 1.

I. Physiological absence of endogenous estrogen stimulation before the puberty.

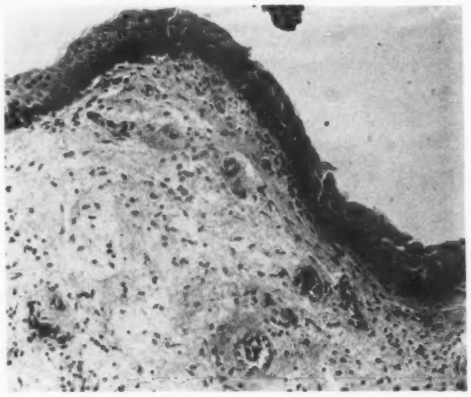


Young female children with resting mucous membrane.

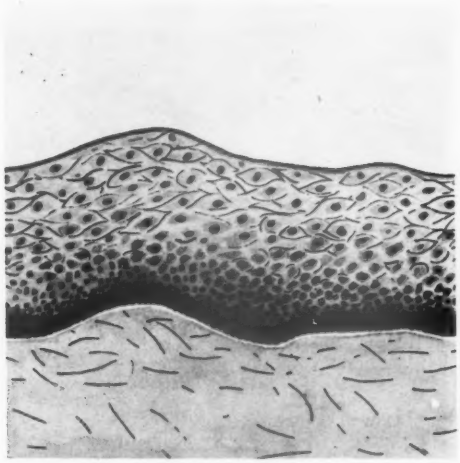


Female children before the onset of the menarche.

II. Physiological deficiency of endogenous estrogen stimulation.



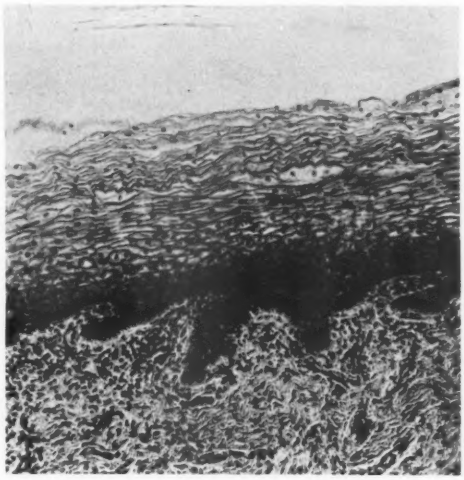
Pregnant women 35 years of age. Abdominal hysterotomy at the end of pregnancy. Posterior fornix of the vagina. (Magnification 6 x 10)



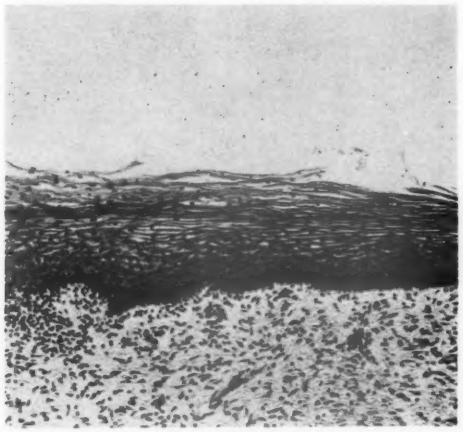
Pubent women with unstable cycle. Hypoplasia of genitals.

III. Physiological presence of endogenous estrogen stimulation.

1. Pubent women with stable cycle.
   Proliferative phase.



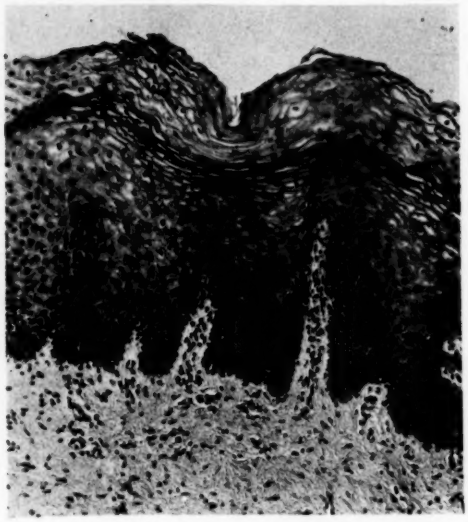
Vaginal epithelium



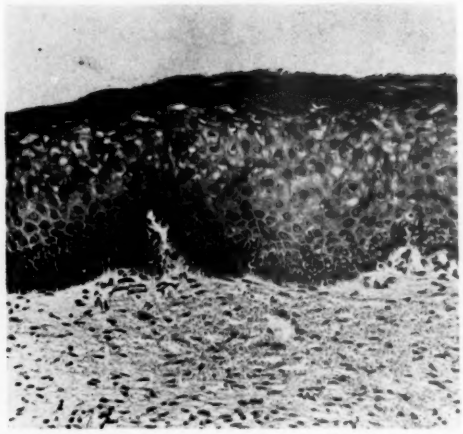
Portio epithelium

40 years of age. Regular cycle. 14th day. (6 x 10)

2. Pubent women with stable cycle.
End of the proliferative phase.



46 years of age. Regular cycle. 18th day. Vaginal epithelium. (6 x 10)



34 years of age. Regular cycle. 17th day.
Portio epithelium. (6 x 10)

3. Pubent women with stable cycle.
Secretory phase.

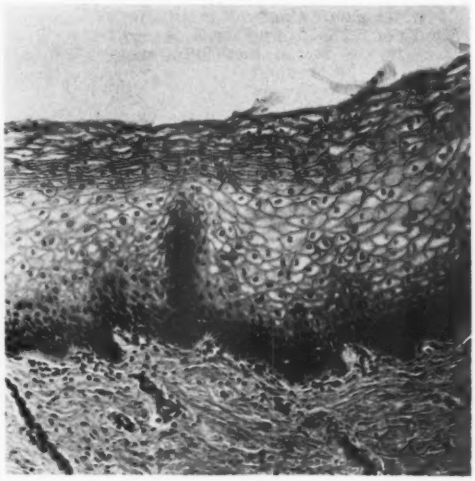
42 years of age.

Regular cycle with intervals of 28-29 days.

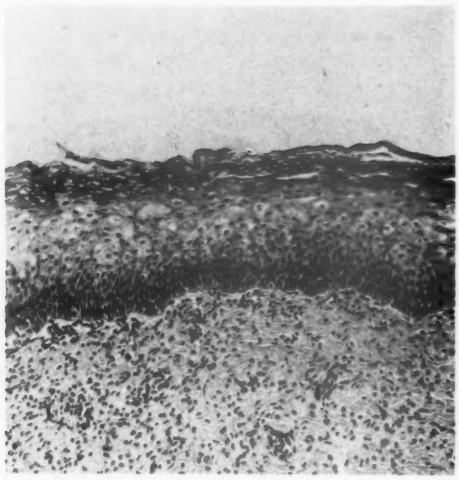
22nd day.

Vaginal epithelium

4. Pubent women with stable cycle.
Late secretory phase. (6 x 10)



Vaginal epithelium

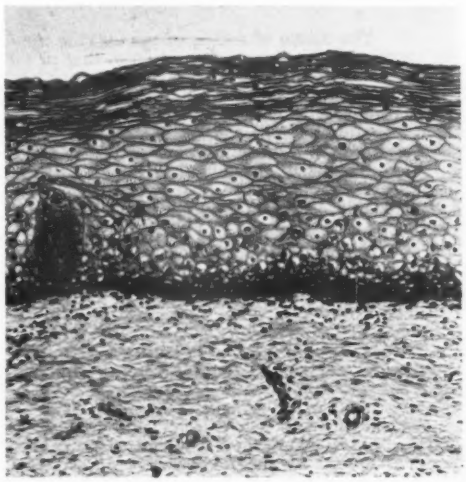


Portio epithelium

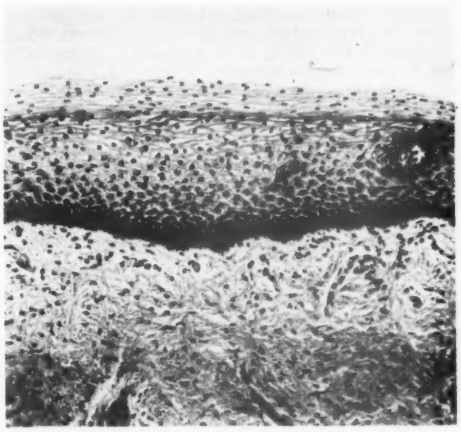
46 years of age. Regular cycle. 24th day. (6 x 10)

IV. Physiological deficiency of endogenous estrogen stimulation in the last stage of puberty.

Women in the climacteric and in the early menopause.



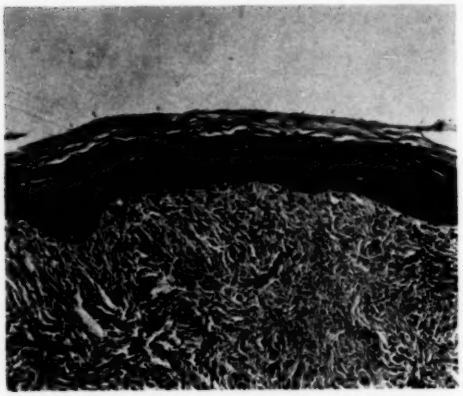
54 years of age. Menopause for the last three years.
Vaginal epithelium. (6 x 10)



54 years of age. Menopause for the last four years.
Vaginal epithelium. (6 x 10)

V. Physiological absence of endogenous estrogen stimulation in the menopause.

Women in the atrophic menopause.



63 years of age.

Menopause for the last 16 years.

Vaginal epithelium. (6 x 10)

SUMMARY

Summarizing, one can say; the estrogen stimulation (estradiol) operates on the vaginal epithelium of human beings during the period between childhood and the menopause. The end organ which reacts at any time in four or five layers to estrogen stimulation exhibits clear histological patterns.

If the hormonal influence is absent, only the germinative zone with a few poorly differentiated cell layers is visible. The stratum germinativum is either hypoplastic, not yet active, but with latent extraordinary growth potential (as in the child) or is atrophic, (or aplastic) as in menopausal women. Certainly, menopausal epithelium can also be stimulated by estrogens and is still able to grow. Whereas one knows or can estimate in the child the period of the future operation, I know nothing about the possible duration of pharmaceutically-produced growth processes of atrophic menopausal epithelium. The time lapse in these cases between individual estrogen secretions is either too great or the amount is not present in sufficient quantities.

Thus a certain deficiency of estradiol affects the maturation of every single vaginal epithelial cell and the whole tissue formation becomes retarded. Every single cell grows older without having reached the degree of maturation and the measurable volume of their "normal" histological prototypes. Only in this way is the deficiency of the gauge of the layers effected. The vaginal epithelium will grow under estrogen deficiency, but not to maturation point. The physiological or fully effective amount of estrogens finally stimulates the proliferation to the known picture of the four- to five-stratified epithelial integuments. During the whole proliferative phase the morphological accent lies on the generative layer which von Herrath considers should be included in the upper limit of the intermediary cells. The relative decrease of estradiol, together with the tissue swelling quality of estriol and corpus-luteum-hormone, affect the sometimes considerable increase of the gauge of the middle and superior layers of the vaginal membrane. One can almost say: the "functional layer" of the vaginal membrane goes from the superior parabasalis - up to the sub-superficialis-cells. What lies above that is desquamated during the whole cycle, but especially at the end of the secretory phase. This superficial layer is continually replaced by the germinative layer.

The scope of duties of the uterine mucous membrane is quite different from that of the vaginal epithelium. Therefore, rigorous comparisons of these two epithelial formations are not possible. The analytical tendency to discuss separately various formations - apart from didactic purposes - must likewise be limited for biological processes, because one may not eliminate the time factor of the evolution and the interrelationship of growth, function and cellular death.

One can always observe the gross regularities even if the appearances of individual epithelial patterns are different due to the above mentioned reasons. The epithelium of the portio vaginalis uteri (ectocervix), which in principle does not deviate from the mentioned mechanisms, should receive a careful consideration. This, however, should not relate to the normal response of this epithelium under estrogen influence, but should relate to the morphological conditions of the subepithelial tissue in the ectocervix of women, who have not delivered, or who have been pregnant one or several times and have delivered.

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NO CLOSING REMARKS

CYTOLOGICAL CHANGES OF THE VAGINAL AND ECTOCERVICAL EPITHELIUM AS A RESULT OF THE PHYSIOLOGICAL PRESENCE, DEFICIENCY OR ABSENCE OF ENDOGENOUS ESTROGEN STIMULATION

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Smears taken simultaneously from various sites (ectocervix, fornices, lateral vaginal walls, outer third of the vagina) from sexually mature women with pronounced FSH- or LH-action, do not, as a rule, show marked differences.

Smears from the outer third of the vagina in women with an atrophic or poorly proliferating vaginal epithelium usually show a higher degree of proliferation than when they are taken from the ectocervix or from the fornices. This

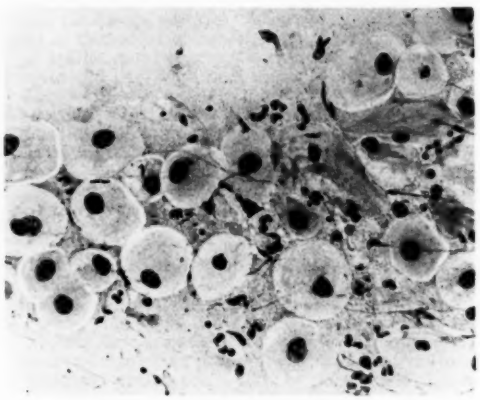

Fig. 1a.

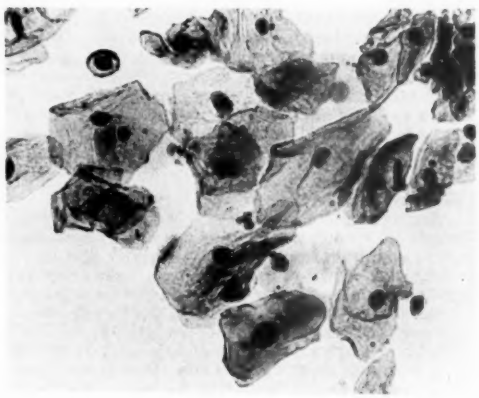

Fig. 1b.

Fig. 1. a) Smear from ectocervix—mainly parabasal cells.
b) Smear from outer third of the vagina—mainly large and small intermediate cells.

(Both specimens from a 63 year old patient, 12 years postmenopausal, taken simultaneously).

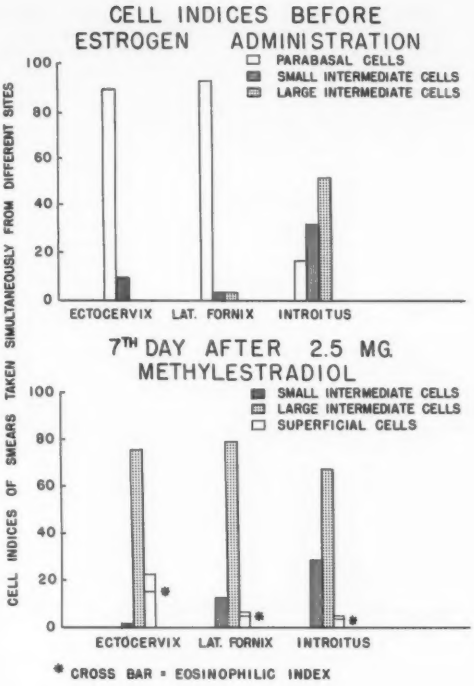

Fig. 2.

more marked proliferation near the introitus is not due to internal, hormonal factors but to external and mainly mechanical factors. An example of the different degrees of proliferation is given in Fig. 1. While the smear from the ectocervix shows mainly parabasal cells, the smear from the outer third of the vagina shows mainly intermediate cells of varying size, as well as a few superficial cells.

When estrogens are administered in low dosage to women with an atrophic vaginal epithelium, one can observe, in many instances, more marked proliferation on the ectocervix than in the lateral fornices, while the outer third of the vagina shows a poorer response. Fig. 2 shows the different cell pictures at the various sites before and after estrogen administration.

The highly proliferated epithelium of the ectocervix has a marked tendency to exfoliate flat, large, single cells; LH action here, therefore, results in less folding and conglomeration of cells than is seen in smears from the lateral fornices.

From these observations it can be deduced that it is of the greatest importance to take smears for hormonal determination always from the same site. The ideal site, undoubtedly, is the upper third of the lateral vaginal wall (lateral fornices) because the epithelium here is least affected by non-hormonal influences.

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Cytological changes resulting from estrogen stimulation were the principal subject of study by Papanicolaou when he made his epoch-making discovery of the exfoliation of malignant cells as a new means of recognizing early and asymptomatic cancer of the cervix. The changes are too well known to be described here again. In addition to Papanicolaou's staining method numerous other methods are now available to demonstrate endogenous estrogen stimulation on the exfoliated cells of the vagina (see Symposium B. ACTA CYTOLOGICA 2: 283-330, 1958). Whichever method one chooses, it is important that the technique remains constant and that the same well-defined cytological criteria are used by all observers if results are to be compared (1). In a later contribution to this symposium we have set forth our own technique and our own terminology with regard to the hormonal evaluation of vaginal smears. We have found it simple and easy to teach, and easily interpreted by the practicing physician.

The physiological presence of endogenous stimulation may be characterized briefly by the predominance of well-differentiated superficial cells. At the highest level of estrogen stimulation the cells are in the majority acidophilic with small pyknotic nuclei. Mild estrogen deficiency can be recognized by predominance of cyanophilic superficial cells which lose their pyknotic nuclei and by an increased number of intermediate cells. In severe deficiency of endogenous estrogen stimulation the number of eosinophilic pyknotic cells declines sharply, while parabasal and cyanophilic intermediate cells represent the bulk of the exfoliated cells. Complete absence of endogenous estrogen stimulation is characterized by marked predominance of basal and parabasal cells with complete disappearance of eosiniphilic superficial cells. Differentiation of the parabasal cells proceeds in irregular and grotesque fashion, leading to many atypical cell forms characterized as involutional dysplasia. In advanced vaginal atrophy with kraurosis small, highly keratinized cells appear to which we have given the name "kraurosis cells." We consider complete absence of endogenous estrogen stimulation in a woman below the age of 60 definitely pathological. Even in cases of bilateral ovariectomy sufficient estrogenic steroids are produced by the adrenal to maintain a certain degree of endogenous estrogen stimulation.

The presence, deficiency or absence of endogenous estrogen stimulation in normal women and in women with numerous gynecological disorders is tabulated in Table I. Over 50% of the vaginal smears of non-menstruating girls in the age group of 10-14 years showed 1-2 plus estrus, but only one-third showed a 3 plus estrus. In menstruating girls of the same age 70% showed

3 plus estrus. In normal menstruating women endogenous estrogen stimulation was highest in the third postmenstrual week, the lowest during menstruation or the period immediately following it. We have not observed a single woman with normal menstruation who did not show some evidence of estrogen stimulation in the vaginal smear. In 98 cases of juvenile amenorrhea between the ages of 16 and 24 years, one-third showed complete lack of estrogen stimulation. One hundred and seventy cases of adult type of amenorrhea were women who became amenorrheic after they had menstruated normally for some time; 82 % showed some type of estrogen stimulation. Women complaining of oligomenorrhea showed in the majority of cases some estrogen deficiency, while women complaining of menorrhagia resembled hormonally normal menstruating women. In 132 cases of sterility with apparently normal menstruation, 50% showed mild estrogenic deficiency. Seventy percent of the women in surgical menopause up to 40 years of age maintained fairly good evidence of continued estrogen stimulation. We could not ascertain whether this was due to remaining ovarian tissue. Eighty-five percent of vaginal smears of women in early menopause up to 60 years of age showed some degree of estrogen stimulation, while estrogen deficiency became much more marked in the vaginal smears of women over 60 years of age. However, even in this group 3 plus estrus smear was occasionally observed. Women suffering from menopausal metrorrhagia appeared no different hormonally from other menopausal women. Vaginal smears from patients with carcinoma in situ of the cervix showed a slight decline in estrogen stimulation, while those from patients with invasive carcinoma of the cervix showed a definite decrease in the estrogen stimulation. This phenomenon had been observed by us during our experimental production of carcinoma of the cervix (2).

Forty-five cases of carcinoma of the endometrium showed a surprisingly high estrogen stimulation as compared with a similar group of menopausal women without carcinoma. In fact, this difference has been so striking in our experience that we hesitate to diagnose an endometrial carcinoma in a woman without evidence of estrus stimulation in her vaginal smear. Seventy-eight percent of our endometrial carcin-

TABLE I

The Presence, Deficiency or Absence of

Endogenous Estrogen Stimulation in the Vaginal Smear of 9000 Women

Menstrual and other gynecological data	Age groups	Total number cases	Classification of estrogen effect in vaginal smear (percentage distribution)			
			zero	1 plus	2 plus	3 plus
Girls, nonmenstruating	10-14	154	6	17	46	31
Girls, menstruating	10-14	27	0	8	22	70
Normal menstruating women, 1st week	20-45	1160	0	5	48	47
" " " 2nd week	20-45	1559	0	0	12	88
" " " 3rd week	20-45	1857	0	0	4	96
" " " 4th week	20-45	1621	0	2	35	63
Amenorrhea, juvenile type	16-24	98	31	40	29	0
Amenorrhea, adult type	30-40	171	18	24	31	27
Oligohemorrhea	22-38	208	0	31	51	18
Menorrhagia	18-35	319	0	3	9	88
Sterility with normal menstruation	20-32	132	0	12	31	57
Menopause (surgical)	24-40	289	8	19	30	43
Menopause (early)	45-60	465	15	27	37	21
Menopause (late)	61-85	452	31	53	12	4
Metrorrhagia (during menopause)	52-72	130	21	38	26	15
Carcinoma in situ of cervix	29-45	178	2	9	17	72
Invasive carcinoma of cervix	36-61	135	5	21	53	21
Carcinoma of endometrium	59-75	45	12	10	41	37

oma group showed an estrus corresponding with that of normal menstruating women, while only 58% of the early menopausal group and 16% of the late menopausal group showed a similar estrogen stimulation. This observation is in contrast to Wied's data (3) as published in the symposium on "Endometrial Cytology and Endocrinology" and in agreement with Graham (4) and Wachtel (5), and also has been emphasized recently by Berg and Durfee (6). Wied has suggested pooling of our material because of the relatively small series each observer has to present and to apply one technique and one set of criteria to this pooled material. We heartily welcome such a suggestion. We have learned to suspect endometrial cancer or endometrial carcinoma in situ in any postmenopausal woman with a high estrogen smear and would like to be shown whether this assumption is incorrect.

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The question as to whether or not the cytological response to estrogen is identical in the cervical and vaginal epithelium can be investigated by comparing smears taken simultaneously from the vagina and the ectocervix. The smears can be classified, according to the cell types found, into mature, intermediate and atrophic smears (1). It can be assumed that if the reaction of the two epithelia to the hormone stimulus is the same, the smears taken from the two sites (posterior fornix, i.e., vaginal smear and exocervix, i.e., cervical smear) should always exhibit the same smear type. If, however, one of the epithelia should be more sensitive to estrogen than the other, the two smears will not necessarily be identical. The epithelium already reacting to a small stimulus will then show a smear type indicating some or even maximum growth (intermediate or mature smear), while the smear from the more sluggish epithelium will lag behind and exhibit no growth response (atrophic smear). Studies on two groups of women at entirely different stages of the reproductive life were undertaken: cervical and vaginal smears were prepared; 1. on a group of lactating women at different times of the post-partum months (1, 2), and 2. on women who were past menopause (3). In both studies it was noted that the cervical and vaginal smears did not always exhibit the same cytological picture, but that usually the vaginal smear represented a more advanced maturation of the epithelium. For instance, the smear taken from the ectocervix of a nursing, amenorrheic woman, whose chiled was 1 1/2 months old, was atrophic, while the vaginal smear taken on the same day contained mainly intermediate cells. Smears taken on the same woman 3 months later again demonstrated a more advanced proliferation of the vaginal epithelium: the vaginal smear contained cyanophilic karyopyknotic superficial cells (mature smear) whereas the cervical smear was still intermediate. In both cases the same hormonal stimulus caused a different response in the epithelium of the two sites, the vaginal one responding with a higher growth than the cervical one. This suggests a greater sensitivity of the vaginal epithelium to estrogen stimulation. Such a difference in sensitivity can be recognized in the smears except under the following two conditions: 1. when there is no estrogen stimulation and the smears from both sites remain atrophic and 2. when there is so much estrogen available that even the more sluggish cervical epithelium is stimulated to full growth and both smears become mature. However, in those cases in which the estrogen is not high enough to mature the cervical epithelium completely, smears will differ.

The commonly accepted opinion that only vaginal smears can be used for hormonal evaluation (4) should therefore be reviewed: the fact that the smears from the two sites are not identical can, if critically interpreted, be effectively used for more detailed information on the available hormonal supply. As the vaginal epithelium is sensitive even to small amounts of estrogen, the vaginal smear taken alone indicates whether any estrogen is present. A comparison of the smear types in simultaneously taken vaginal and cervical smears on the other hand, is able to convey information on the amount of available hormone. The examination of the vaginal smear alone plus the comparison of smears from the two sites give different kinds but complementary information relating to the presence, absence or deficiency of estrogen stimulation.

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DISCUSSION

RAYMOND BOURG, Brussels, Belgium:

Although a complete study has not been completed, there is a significant difference between the responses of the vaginal and cervical epithelia to the same dose of estrogens. Moreover, the cervical epithelium shows inflammatory alterations, areas of metaplasia, leukoplakia and dysplasia which interferes with the hormonal interpretation of the epithelium. The hormonal evaluation based on the study of a cervical smear is sometimes possible when none of these modifications are present. The vaginal smear taken from the lateral fornix is preferred to the smear taken from the posterior fornix where various endometrial, cervical and vaginal secretions are collected.

A few more remarks should be made:

1. The study of approximately one hundred vaginal smears obtained from colored women in the Belgian Congo revealed the presence of anuclear squamous elements in the posterior one-third of the vagina. Their origin and significance and correlation with some sexual trauma or excessive washing can be ascertained. It is not a manifestation of estrogenic activity.

2. Castration performed between 40 years of age and physiological menopause practically always followed by atrophy of the vaginal mucosa. Sometimes this atrophy is transitory and is followed by a compensatory steroid activity. Treatment with natural or synthetic estrogens should be given in every case of vaginal atrophy in order to supply the minimal estrogenic level which is required and is normally present. The absence of this minimal estrogenic activity is the source of multiple and various pathological conditions.

3. A particular type of vaginal smear is observed among long-time castrated or post-menopausal women: the pseudo-pregnancy smear. This type of smear shows intermediate folded navicular cells in clusters. Personally I believe that this type of smear is not the result of progestational activity but reveals estrogenic deficiency.

ZELMA A. KALNINS, Winston-Salem, North Carolina, U.S.A.:

Dr. von Haam has made an excellent evaluation of cytological findings according to endogenous estrogen stimulation in different physiological and pathological conditions.

I regret that not being familiar with his criteria for classification of estrogen effect in vaginal smears, I am not able to statistically compare our findings with his.

Bichenbach and Soost and Hannah Peters arrived at different conclusions concerning cytological response of estrogen stimulation in women with atrophic epithelium. Bickenbach and Soost observed more marked proliferation of ectocervical epithelium, while Hannah Peters observed more advanced maturation of vaginal epithelium.

While such controversial opinions exist, I would hesitate to make an estimation of the amount of estrogen present from differences between cytological pictures of vaginal and cervical smears.

GILDARDO LEGORRETA, Mexico, D.F., Mexico:

I agree with Hannah Peters in that the ectocervix reflects more sluggishly the action of estrogens than does the fornix. I find quite interesting her suggestion of using the different responses of fornix and cervix as a means of obtaining more information on the endogenous estrogens.

Although in different degrees, both cervical and vaginal epithelia reflect the action of the unopposed endogenous estrogens, and the changes may be observed in the smear in the form pointed out by von Haam. In studying the deficiency of estrogens, it is very important to take a series of smears over a time proportional to the duration of the disorder.

It should be remembered that steroid hormones other than estrogens can produce a proliferation of the intermediate type either from an atrophic epithelium or from a regression of a mature (estrogenic) epithelium.

LUIS MONTALVO RUIZ, Madrid, Spain:

Our observations on the changes of ectocervical and vaginal epitheila as the result of endogenous estrogenic stimulation, more or less completely agree with the statements of the authors, as we have noted in various papers (1, 2, 3).

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CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

To complete the remarks for discussion a report of some of the details of our examination might be allowed. We took smears from 250 women of different ages and with different hormonal status. The smears were taken simultaneously from the ectocervix, the posterior vaginal fornix, the upper third of the lateral vaginal wall and the external third of the vagina. One can note the frequency of equally formed cell patterns by examining Table I.

There is no doubt that the higher proliferation of the vaginal epithelium in the area of the introitus is not caused by hormonal influence. The proof for this fact can be demonstrated by administration of estrogens. The epithelium in the area of the introitus reacts poorly. This is shown in Figures 2a and 2b of our paper, where we counted the Cell-Indices in the smears before and after administration of estrogen.

The cause of the higher proliferation at the ectocervix remains obscure to us. It appears mainly in specimens with cell patterns of intermediate proliferation, poorly indicating moderate estrogenic effect. It seldom appeared when the smears from the lateral vaginal wall showed marked estrogenic effect. It never appeared in the presence of an atrophic vaginal epithelium. The fact that we observed a higher proliferation at the ectocervix in some cases after

administration of low dosages of estrogens, seems to show rather that the ectocervical epithelium has a special sensitivity toward hormonal stimuli. On the other hand, one must consider that chemical influences by the cervical mucus and abnormal regenerative changes in areas of transformation zones may play some part.

In a certain number of cases in the smears taken from the ectocervix we observed the appearance of parabasal cells in addition to the superficial cells and large intermediate cells, whereas, these cells were not found in the smears from the lateral vaginal wall. The parabasal cells mostly appeared isolated, but sometimes also in large clusters. As observation showed in these cases, there was always present an ectopy, an "erosion vera" or at least a tiny laceration recognizable under the colposcope. We dealt actually with local disturbances. It certainly would be incorrect to conclude from such findings on decreased estrogen effect on the ectocervix. We could talk here about a "pseudohypofolliculinia."

In no case did we find a uniformly lower proliferated cell pattern on the ectocervix in women with marked estrogenic effect. In cases with an endocervically located squamo-columnar junction one could observe occasionally smears from the external third of the cervical canal; that there was a higher proliferated epithelium present than on the ectocervix, this would also speak against a decrease of hormonal sensitivity at the areas close to the uterus. We only occasionally observed a lower proliferation on the ectocervix in women with marked estrogen deficiency. We are, however, not sure in these cases whether there is really a decreased sensitivity or again a deception by "erosion cells," which can be discerned from lowly proliferated cells with difficulty. It seems that two processes concur here: the higher proliferation of the distal portions of the vagina (which is certainly not hormonally caused) and the higher proliferation on the ectocervix (which is possibly hormonally caused). This may be due to the fact that in some cases the effect of estrogen may seem to be higher at the ectocervix and in the distal third of the vaginal than at the lateral vaginal wall.

One can also see significant differences in folding and crowding of cells in smears prepared from the ectocervix and vaginal. Both crowding and folding are often more marked in smears from the lateral vaginal wall. This also can convey the impression of a more marked estrogenic effect in ectocervical specimens.

The findings of Wied (6) coincide with our observations. Wied established the percentage of superficial cells (old nomenclature), the pyknotic nuclei and the eosinophilic cells from five different spots (one cm. above the introitus, three to four cm. above the introitus, vaginal fornix, ectocervix and cervical canal). Wied found the lowest value in the fornix: SI = 91%, KI=35%, EI=23%. All the indices increased markedly near the introitus as well as close to the ectocervix and cervical canal respectively. Thus, the following Indices resulted one cm. above the introitus: SI=98%, KI = 51%, EI=34%. In the cervical canal they were: SI=91%, KI = 42%, EI = 32%.

Nieburgs (1) mentioned, that bacterial cytolysis is often less in smears taken from the lateral vaginal wall. We, like Pundel, cannot follow his conclusion of taking smears for hormonal diagnosis from the ectocervix rather than from the lateral vaginal wall. If one, however, assumes that the cells from the ectocervix are more mature and, therefore, cannot be affected by Döderlein bacilli as much as the less mature cells exfoliated from the lateral vaginal wall, it would mean a certification of our findings.

The findings of Hannah Peters are in contrast to our results concerning the comparison between ectocervix and vagina (fornix). As far as we know from personal discussions with Hannah Peters and from her publications (2, 3) these examinations were performed (1) on Indian women and (2) exclusively postpartum or during the lactation period. Our material mainly derives from women, who were not pregnant and were not in the puerperium. Only in 29 cases were the smears made within the first six weeks postpartum, but these did not exhibit any basically different criteria either.

Asking for the cause of differences in the findings one has to consider the following possibilities:

1. Are there racial differences?
2. Does one have to consider in the postpartum more often deception by "erosion cells?"
3. Can the increased proliferation of the epithelium of the ectocervix in marked estrogen activity be followed by an especially low proliferation in cases with absence or deficiency of estrogen activity?

We are pleased to conclude that the lateral vaginal wall was acknowledged to be the best place for preparation of specimens for hormonal diagnosis. We, like Kalnins, wish to warn that the difference in cell findings in specimens from the fornix and ectocervix does not allow a quantitative hormonal estimate. Too many non-hormonal influences may play their part in this respect. The different reactions at different sites of the epithelium of the reproductive tract to hormonal stimuli is of great importance since they may be the cause of misjudgement in hormonal evaluations on these points.

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EMMERICH von HAAM:

I agree with Bourg in recommending estrogenic therapy in women with vaginal atrophy, although I usually limit my recommendation to women below the age of 70.

CYTOLOGICAL CHANGES OF THE ENDOCERVICAL EPITHELIUM AS A RESULT OF THE PHYSIOLOGICAL PRESENCE, DEFICIENCY OR ABSENCE OF ENDOGENOUS ESTROGEN STIMULATION

JEAN A. de BRUX and

HENRIETTE WENNER-MANGEN

Paris, France and Luxembourg, Luxembourg

I. Histology

The endocervix is lined with a columnar, unistratified, muciparous mucosa. The structure, form, abundance and dimensions of the glandular tubes vary according to the genital activity, and also from one individual to another.

The epithelium is composed principally of three cellular types:

1. the functional element, a tall cell (30 μ - 40 μ), very narrow, measuring 4 μ at its base of implantation, and particularly numerous in the glands;
2. the quiescent cell, shorter, somewhat prismatic in form; and
3. the ciliated cell and that having a single cilium, with thickened cytoplasmic membrane.

The latter varieties may be found interposed among secreting cells, but are most often found in groups at the surface of the folds.

The three morphological types, together with the transitional types, represent different stages in the evolution of the same element. The position of the nucleus and the abundance of the cytoplasm depend on the functional state of the cell.

Reserve cells are found in sub-columnar position. They are round or slightly polygonal, clear, and of undistinguished appearance. (They should not be confused with the endometrial clear cells, which, according to Feyrter and J. H. Müller, have a hormonal activity as yet undefined).

The endocervix has a connective stroma, stocked with fibrocytes and having a fine argentaffine web surrounding the glands.

In the upper two-thirds of the endocervix, the glands, more numerous, assume a purely tubular aspect.

The isthmus is a zone of transition. Here, the epithelium is less high (20 μ - 30 μ), and only occasionally ciliated. The cytoplasm is dark, foamy and granular, and the nucleus is usually median.

II. Histophysiology

The existence and mechanism of a histological cycle parallel to the estrual cycle is subject to discussion. Nevertheless, the changes in morphological aspects coinciding with the different periods of ovarian activity would seem to bear out the idea of a hormonal influence.

In the prenatal period, the endocervix develops exuberantly. The glands are numerous and very ramified, and the epithelium is hypertrophic. An ectropion frequently exists. This state is determined by the maternal hormones.

Shortly after birth, the endocervix undergoes an involution. The glands shrink and become smooth, the epithelium is low and quiescent. The endocervix conserves this aspect until puberty.

After the menopause, the mucosa thins. The stroma becomes dense, and later sclerotic. The glands disappear progressively.

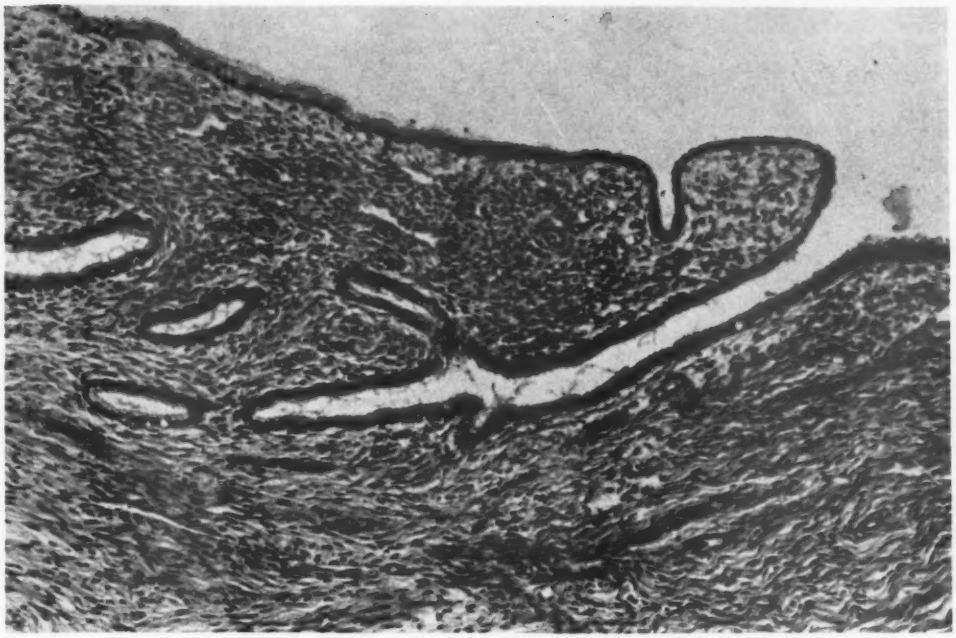

Fig. 1. Endocervical epithelium: Deficiency of estrogenic stimulation.

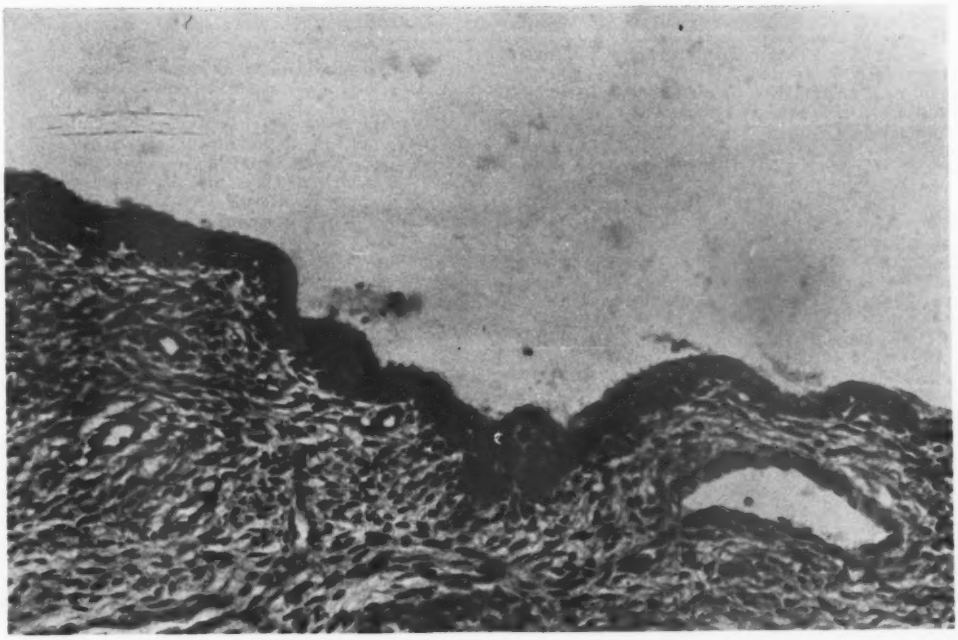

Fig. 2. Endocervical epithelium: Very low level of estrogenic stimulation with beginning metaplasia.

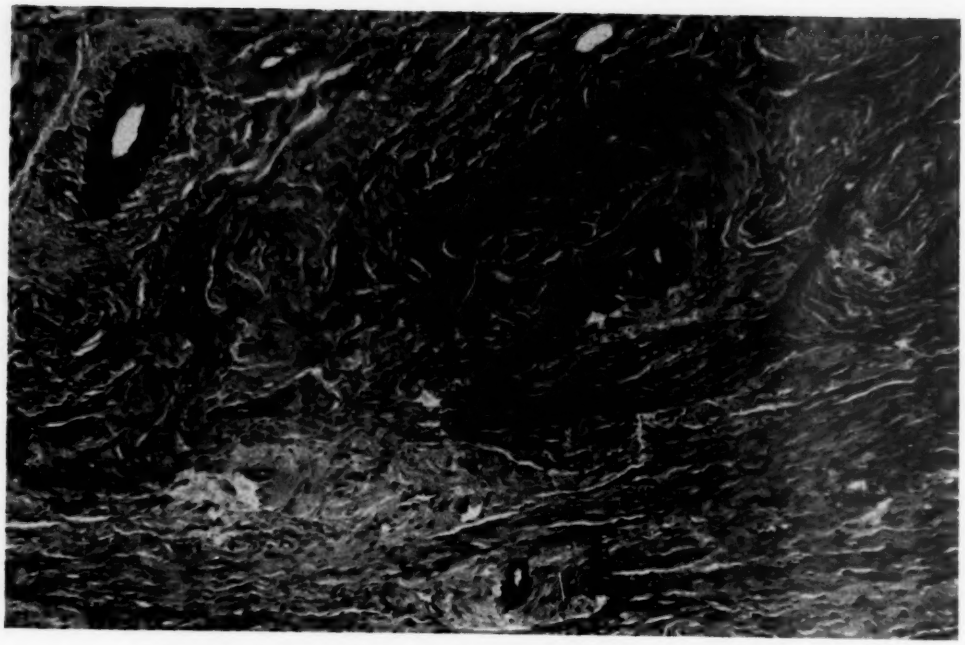

Fig. 3. Endocervical epithelium: Absence of estrogenic stimulation. Disappearance of functional glandular cells, atrophic glands and sclerosis of the stroma.

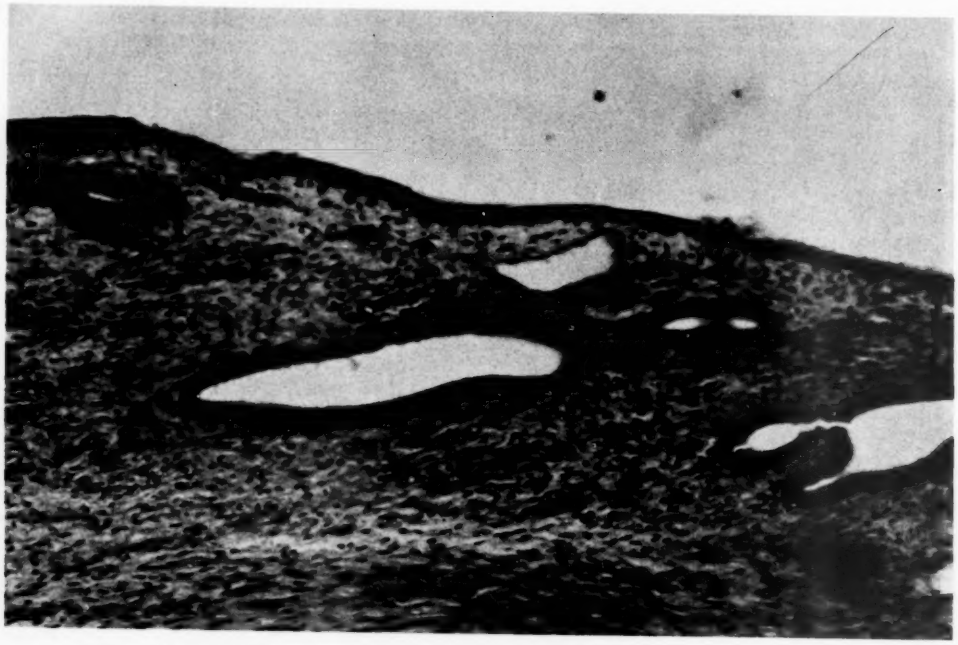

Fig. 4. Stroma of the cervix during menopause: Fully developed sclerosis and stenosis of the vascular lumina.

The epithelium is uniformly low and composed solely of undistinguished and non-secreting prismatic cells. In the castrated woman this picture makes its appearance more rapidly (Figs. 1, 2 and 3).

Cyclic variations of the endocervical glands

The notion of the existence and mechanism of clearly characterized cyclic endocervical variations at the histological level remains as yet little understood and controversial. Such modifications, denied by Schroeder, Topkins, Duperroy, et al., have been affirmed by Wollner, Sjovall, Guillon, Papanicolaou, Traut and Marchetti, Tietze, and Palmer, but their data are not in agreement, either on the morphological criteria and their determinism, or as concerns the periodicity and duration of the different phases.

Wollner describes a proliferative phase (with rising of the nucleus and edema of the stroma), and a secretory and desquamative phase, synchronous with those of the endometrium.

Sjovall declares the existence of a secretory phase from the 12th to the 22nd day, characterized by the appearance of papillary excrescences. He has observed no nuclear displacement nor catamenial exfoliation.

Papanicolaou, Traut and Marchetti describe modifications synchronous with those of Sjovall.

Topkins finds very wide individual variations, but independent of the cycle.

Palmer and Guillon distinguish:

1. a stage of progressive stimulation beginning just after the menses and lasting until the 14th day;
2. a stage immediately preceding the excretion;
3. the expulsion, in which flakes of mucus appear in the lumen of the glands;
4. a quiescent period, with infiltration of leukocytes; and
5. toward the 24th or 25th day, a resumption of reduced secretory activity coinciding with the premenstrual follicular peak.

III. Cytology

A. Cytological modifications induced by the estrogens during the menstrual cycle.

1. Follicular phase:

During this phase there may be distinguished several stages:

a. Proliferative stage: During the days following the menses, the endocervical epithelium is relatively low and uniform. The cells are at rest, and the nucleus is basal.

The 9th or 10th day the cells begin to proliferate (probably by amitosis). They become hypertrophied and reach their maximum height (35 μ - 40 μ), but remain narrow (4 μ). The nucleus is elongated, basal or submedian, finely and regularly granular, and contains a single, small, chromatic nucleolus. The cytoplasm, whose intercellular limits are indistinct, shows a clean-cut apical border, regular or finely notched. Secretory activity is reduced or absent. Wedged in among cells of this type, there are found rare neutral cylindrical or cuboid cells.

b. Secretory stage: From the 10th to the 12th day, mucus accumulates progressively at the apical pole of the cell. If the charge is great, the apex protrudes at the surface of the epithelium. The cell becomes wider and slightly flattened, and the nucleus, median at the beginning of this stage, is pushed toward the base. If the mucus is in large quantity, the nucleus may become deformed and the mucus invade its lateral portions (goblet cells).

This stage immediately precedes the excretion and coincides with the lowest point of the basal temperature. At this stage, Palmer and Guillon have observed in the nuclei numerous images of cleavage, which seem to occur obliquely in relation to the nuclear axis.

c. Excretory stage: From the 12th to the 16th day. This long duration of excretion is explained by the fact that all the cells do not reach maturity within the same lapse of time. The cells are of medium dimensions. The apical pole, intensely mucicarminophile, is indistinct and gradually disappears in the mucus. The nucleus rises and becomes median or submedian. It is large and clear, with reduced chromatin irregularly distributed and having a tendency to pack against the nuclear membrane. The nucleolus, which is hypertrophic, may be multiple.

2. Luteal phase

Toward the 17th to the 18th day, the epithelium loses all functional character and begins its regression. It flattens out and the cells sometimes then become ciliated. These may either be multiciliated or have a single cilium: They are low, and have an exceptionally thick cytoplasmic membrane. The cytoplasm of the cells at rest contains numerous mitochondria, which give it a granular, dark aspect. There is an infiltration of leukocytes noted.

Sometimes, toward the 25th day, and coinciding with the premenstrual eosinophilic peak observed in colpocytology, one may observe a slight, transitory resumption of secretory activity.

From the standpoint of periodicity, the endocervical secretory activity - destined to realize the conditions favorable to the ascension of the spermatozoa during the period when fecundation is possible - precedes the endometrial secretory transformation which favors nidation.

3. The cervical mucus: The macroscopic, microscopic and, particularly, the rheological properties (elasticity, plasticity, runniness) are determined directly by the folliculin.

a. Macroscopic modifications: The estrogens provoke the appearance of an abundant (x 10), transparent (by reason of its high water content: 98%), and very runny glair.

With a partial deficiency of folliculin,

the glair is scanty, opaque and much less runny.

With total folliculin deficiency, the glair is absent or extremely rare, whitish, and very viscous and sticky.

b. Microscopic aspect: The number, aspect and grouping of the cells is in relation to the charge of estrogens. Their presence diminishes the number of endocervical cells and polynuclears. In the ovulatory phase one finds one to five per field (x40).

The cessation of the estrogenic stimulation determines an increase in the number of endocervical cells and polynuclears proportional to the degree of deficiency. If the stimulation is absent, one may count 20 - 40 elements per field.

According to Pundel, the exfoliated endocervical cells diminish in number at the beginning of the charging of estrogens. At this stage (beginning follicular stage), they are isolated and oval in form. Paralleling the appearance of an endocervical glair, he observes a more abundant exfoliation. The endocervical cells, isolated and in sheets, oval or cylindrical, often contain vacuoles. The nuclei are usually round. One may find a considerable number of free nuclei, particularly during the period of maximum secretion. Contrary to Palmer's observations, Pundel describes a decrease in the number of endocervical cells following ovulation.

B. The hormonal causes of the cytological variations

The proliferative activity and the secretory transformation of the endocervical cells is determined exclusively by the folliculin. The expulsion of the mucus is a mechanical phenomenon.

After cessation of the estrogenic action, the epithelium loses its functional activity and begins to regress. In a first stage, there are numerous signs of degeneration: pyknosis, karyorrhexis, vacuolization of the nuclei. The cells are eliminated into the lumen of the glands. After this, the epithelium takes on the neutral appearance of a lining: the cells are low, uniform, trapezoid, and with or without cilia.

The cytological modifications observed at the beginning of the cycle may be integrally reproduced artificially.

The administration of estrogens (2 - 5 mg. according to the duration of the deficiency) determines in 24 to 48 hours a hypertrophy and secretory transformation of the endocervical epithelium, with appearance of a noticeable cervical glair.

The involution of the mucosa during the second half of the cycle in spite of the persistent high degree of estrogenic stimulation coincides with the first signs of activity of the corpus luteum, which has an inhibitory action on the endocervical epithelium. This restraining effect of the progesterone may be demonstrated by administering progesterone to a castrated woman having follicular charge. The cervical glair disappears and the epithelium regresses. The inhibitory ratios of progesterone/estrogen (P/E) have been studied by Palmer, who injected estrogens and progesterone simultaneously:

The ratio P/E 5/2 determined a partial inhibition.
The ratio P/E 10/2 determined a total inhibition.

These data are valuable for an understanding of the mechanism of certain hyperplasias, metaplasias and dysplasias of pregnancy, in which these ratios may be disturbed.

The study of the role of the estrogens in the development and secretory charging of the endocervical glands may also be approached by the tracers. The radio-isotopes - in particular S 35 - permit the topographical study of the synthesis of the mucins from sulfuric mucoitin acid. Moricard and Gothie have shown a concentration of S 35 in the endocervical epithelium in the guinea-pig in estrus, in the castrated guinea-pig having received 2.5 mg. of estrogens, and in human endocervical glands in vitro.

C. Modifications induced by abnormally high or unbalanced secretion of estrogens

During pregnancy one may frequently observe modifications - particularly hyperplastic - of the mucosa related to a high and prolonged degree of estrogens, insufficiently or irregularly inhibited by progesterone. The epithelial hyperplasia is manifested by the increased number of the glands, their dimensions and their tortuosity. They become irregular, with numerous polypoid cellular projections.

If the hormonal disequilibrium is marked, the epithelium tends to lose its glandular character. The cells are shorter, the nucleus becomes median, and the secretion diminishes. With a more advanced degree, the epithelium tends to become multistratified. At first, this is a multistratification of undifferentiated reserve-cells. If the incitation continues, there is observed a differentiation, with the characters of a normal or dysplastic epidermoid epithelium. This form of metaplasia induced by the estrogens, if it terminates in a total or excessive maturation, is also designated by Fluhmann as "prosoplasia." It may be reproduced experimentally.

An additional argument relative to the role of the estrogens in the constitution of the modifications of the epithelium is represented by the anomalies of the same type observed in menopausal women treated with high or prolonged doses of follicular hormone.

The same images are found in patients having a feminizing ovarian tumor.

HORST SMOLKA
Kiel, Germany

During puberty the glands of the endocervical epithelium show marked ramifications and protrusions as well as a relatively great number of tall cells. On the other hand, the postmenopausal sections present predominantly glands of

Fig. 1. Endocervical glands with marked ramifications and protrusions. Puber woman, 44 years of age. (Obj. 20x, Ocul. 10x)

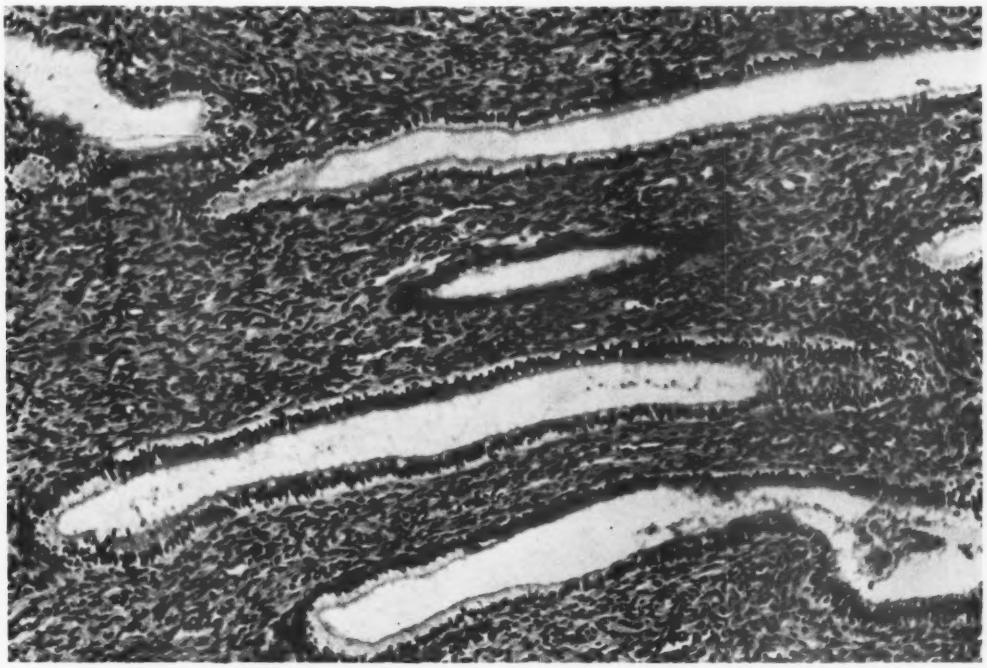

Fig. 2. Straight tubes of gland of the endocervix with relatively short columnar cells. Postmenopausal woman, 70 years old. (Obj. 10x, Ocul. 10x)

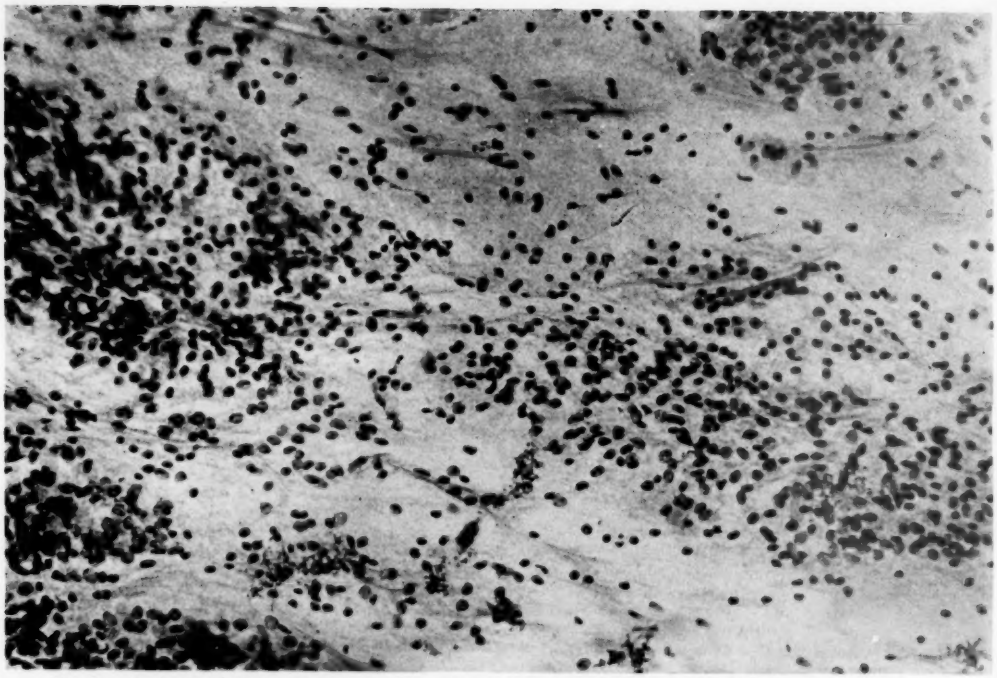

Fig. 3. Typical endocervical smear in the middle of the cycle (14th day). Great numbers of free nuclei of plasmolyzed columnar cells in mucus. (Obj. 10x, Ocul. 10x)

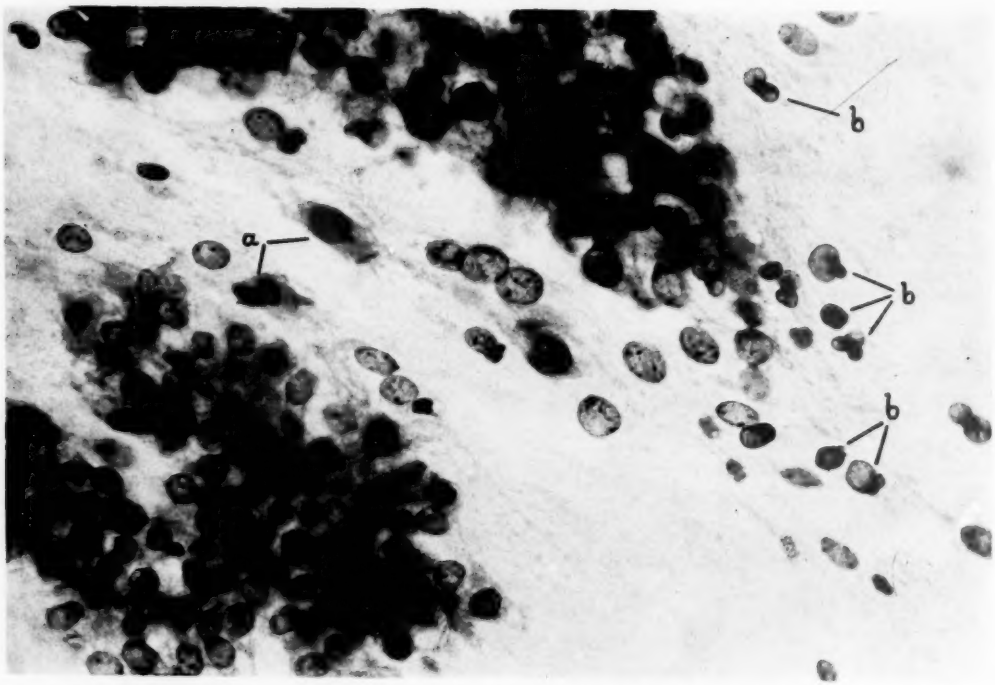

Fig. 4. Plasmolyzed secretory cells of the endocervix. a. ciliated cells. b. free degenerated nuclei of ciliated cells. (Obj. 40 x oil, Ocul. 10x)

a straighter shape or with less protrusions, and the columnar cells are mostly a little shorter. The cause of these alterations is the deficiency or absence of estrogen. In comparison with these alterations, the changes of the epithelial structure and the morphology of the cells during her reproductive period are much less pronounced which seems to be also confirmed by the fact that the histological studies in this respect have resulted in contradictory findings (1-5, 7-10, 12, 13). In the normal cycle there are, however, striking variations in the quantity and quality of the mucus produced by the endocervical secretory cells.

We have examined endocervical specimens of 322 patients and studied the cyclic changes. It has been found that the smears contained more mucus in the middle of the cycle. There was a distinct increase in the number of leukocytes during the menstrual phase. After a marked decrease in the early follicular phase the number of leukocytes increased again in the course of the late follicular phase.

The different forms of the secretory cells (short/broad, medium-sized, long, slim) could not be co-ordinated with the different cyclic phases. Ciliated cells were found in the smears at any time of the cycle. Any regular variations in their numbers were not observed.

In the middle of the cycle the cells were, for the most part, not well-preserved. In the late follicular phase and until some time in the early luteal phase, we found an increased number of free nuclei. The result of comparative studies was that the cells were generally better preserved during menopause than in cases of pathological estrogen hyperactivity. This may be regarded as a certain parallel to the cyclic phases, and it can be concluded that in case of an increased activity of the follicle stimulating hormone there is an increase in the exfoliation of columnar cells. The cells undergo plasmolysis relatively soon, causing the presence of free nuclei. This last-mentioned observation corresponds to the results reported by Papanicolaou (6) and Papanicolaou, Traut, and Marchetti (7). On the other hand, we have observed neither alterations in the location of the nucleus within the cell nor other significant morphological changes of the cells during the different cyclic phases.

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DISCUSSION

LEOPOLD G. KOSS, New York, New York, U. S. A.:

Both above authors have discussed the histology and cytology of the endocervical mucosa at considerable length, but both failed to point out one diagnostically significant alteration occurring in the nuclei of endocervical cells at the time of ovulation: at this time the nuclei produce a short cyanophilic, nipple-like protrusion which is commonly referred to in this laboratory as the "endocervical nuclear tit." This "tit" is so beautifully shown in Smolka's photograph, #4, (the central letter b), that no additional pictures appear necessary. The "tit" corresponds to the peak of estrogenic activity and therefore is of diagnostic importance; it has been previously observed by Papanicolaou and is mentioned in his Atlas. The mechanism of its formation is unknown to the writer. Grace Durfee has also observed this phenomenon following administration of estrogens to postmenopausal women.

A word about ciliated endocervical cells: de Brux and Henriette Werner-Mangen apparently suggest that such cells may be found over the entire endocervical surface. I do not believe that this is so. Möllendorff suggests that the only location of ciliated endocervical cells is in the upper portion of the endocervical canal near the internal os and this is the only area where this discussor observed such cells. Also, the writer has never seen an endocervical cell with a single cilium. May we have a picture, please? De Brux and Henriette Werner-Mangen also state that the endocervical cells in the luteal phase "sometimes become ciliated." I do not believe that this is biologically possible. A cell cannot "become ciliated" in the luteal phase. Cilia are an expression of a very high degree of cellular differentiation and one would have to assume that the endocervical cells which, incidentally, have a very low turn-over, would lose the cilia at the beginning of the next cycle and the whole cycle would start all over again. This is most unlikely and this discussor is tempted to believe that strands of mucus may be sometimes confused with cilia. Only cells with a thick terminal plate may be considered as ciliated. The other "cilia" do no count.

De Brux and Henriette Werner-Mangen also state that endocervical cells proliferate "probably by amitosis." This would be most extraordinary indeed. There is no proof whatever that amitotic divisions may occur in the human

body. Mitoses may be observed in the endocervix, but even in their absence it does not appear justified to suggest amitotic division of cells.

This discussor does not understand de Brux' statement that the inhibitory activity of the progesterone/estrogen ratio "is valuable for an understanding of the mechanism of certain hyperplasias, metaplasias, and dysplasias of pregnancy in which these ratios may be disturbed." Unless there is a translation defect this just doesn't make much sense. Would de Brux believe per chance that "dysplasias" of the cervix - lesions with a neoplastic slant - are due to a hormone imbalance? An explanatory note would be much appreciated.

Finally, Fluhman's "prosoplasia" (incidentally the term was used many a time before Fluhman), was a purely experimental situation. There is no proof whatever that the squamous metaplasia of the human endocervix is due to an excessive estrogenic activity.

CLOSING REMARKS

JEAN A. de BRUX:

The observations of Möllendorff and Schröder concerning the localization of ciliated cells in the endocervix no longer seem to be confirmed by contemporary research.

In fact, although the upper third of the endocervix is lined solely by ciliated cells, the lower two-thirds contain these cells interspersed among the mucus-secreting cells. Their number and localization are determined by the menstrual cycle (1, 2).

Nevertheless, some authors, in particular Guillon (3), consider that the columnar cells of the epithelium lining the endocervix are devoid of cilia, that there exists some kind of mucoid secretion similar to that seen in the stomach, and that only the glandular cells are ciliated. This latter assertion does not seem to be verified.

Transformation of the ciliated cells into mucus cells:

Prenant, cited by Branca and Verne (4) in their treatise on histology refers to an "alternation of evolution of the ciliated cells into muciparous cells." Dubreuil (5), Guillon (3), Duperroy (6) and more recently Schüller (after a phase-microscopic study), believe that the ciliated and the muciparous characters are different stages in the life of the endocervical cell. This moreover, is a well known phenomenon of general biology at the level of the digestive tract, the respiratory tract and the Fallopian tubes.

As for the endocervical cell with a single cilium, its existence is affirmed by Dubreuil and Baudrimont. I personally have never encountered it.

The "nuclear tit" is new to me only in so far as this designation for it is concerned. In 1944, Guillon noticed and described at length this modification of the nuclei during the charging-up period (folliculinic action) of the endocervical cells. He interprets this aspect as an image of cleavage, and describes it thus:

> "There are found in the nuclei of the glandular cells numerous images of cleavage, which seem to occur obliquely to the nuclear axis, but it is impossible to determine either the precise moment at which the cleavage takes place, or that at which it is prepared."

It is possible that this image cannot be called amitoses, but it is consoling to note that Koss and Grace Durfee have no explanation to offer for this phenomenon.

However, the phenomenon of amitosis occurs relatively often in the organism, even aside from tumors. Borst, Maximow (7), and Altschul (8) have observed it in connection with cells of the connective tissue. More recently, Crusdew (9) has observed direct divisions of epithelial cells in the postpartum endometrium.

Finally, I regret that my phraseology was too fore-shortened to permit the understanding of my thought concerning the inhibitory action of progesterone on the action of the estrogens according to a well-established ratio (10). Since progesterone exerts an inhibitory influence on estrogenic activity, we might deduce from this that a disturbance of this balance could be the origin of the metaplasias and dysplasias (especially during pregnancy), which, in fact, translate a hormonal imbalance.

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HISTO- AND CYTOCHEMISTRY OF BASAL AND PARABASAL CELLS

CONSTANTIN HEROVICI
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I. INTRODUCTION

The aim of this study is to histochemically characterize vaginal cells and further observe if these characteristics undergo cyclic modification under the effect of hormonal stimulation.

A. Material of Study

The histochemical study has been effectuated by the use of vaginal scrapings and vaginal biopsies.

The scrapings have been taken the eighth, fourteenth, and twenty-fourth days of the cycles of ten menstruating women, as well as from ten women ten years after their menopause. These latter had no superficial cells in their samples.

The vaginal scrapings were fixed in ether-alcohol, and the vaginal biopsies in formal-acetic-alcohol.

We have studied the nucleic acids, glycogen, the polysaccharides, the alkaline phosphates, and potassium, as well as the SH groups.

B. Techniques

We used Feulgen's technique for DNA and Brachet's techniques, with personal modifications, to demonstrate the DNA, RNA ratio.

This personal modification consists of adding orange G to the methyl-pyronine, thus permitting differentiation of cytoplasm rich in RNA (red) from cytoplasm having very little (orange). The technique of Brachet (1) is almost quantitative by this personal modification. Controls are carried out by Panigel's method (biliary salts).

1. Glycogen: Best's carmine and iodized rubber controlled by salivary amylase.
2. Polysaccharides: PAS technique described by MacManus and reported by Lison (3.).
3. Alkaline phosphatase: Gomori's technique, Danielli's modification (reported by Lison) (3).
4. Potassium: MacCallum technique, modifications by Marza which demonstrates potassium proteid (Lison) by transformation after alcoholic fixation of sodium cobalt nitrite to potassium and sodium cobalt nitrite, black in color.
5. Group SH: Chevremont and Fredericq test based upon the reduction of ferric ferricyanure to ferric ferrocyanure color, control slider are treated by mono-iodo-acetic acid (4%) (3).

II. THE HISTOCHEMISTRY OF BASAL AND PARABASAL CELLS

A. Nucleic Acids

The deep basal cells are characterized by a high quantity of cytoplasmic RNA, by an equally high quantity of nuclear DNA and a normal amount of nucleolar RNA. In the external basal cells, less DNA and less cytoplasmic RNA are found but there is more nucleolar RNA. The nucleic acids do not seem to participate in the menstrual cycle of women.

B. Glycogen

Faint traces of glycogen are found in the cells of the parabasal layer, but there is a total absence of glycogen in the basal layers. There is no quantative difference in the vaginal scrapings of the eighth, thirteenth, or twenty-fourth day.

C. Polysaccharides

The reaction for polysaccharides is faintly positive in the cells of the parabasal layer; the MacManus reaction is negative in the basal layer.

D. Alkaline Phosphatases

In this layer an intense Gomori reaction is evident, but without modification during the cycle.

E. Potassium

The Marza technique is positive at the level of the nucleus and the cytoplasm of the basal layer and is more intense in the deep layer and in the external layer. It remains constant throughout the cycle.

Sulfhydryllic Groups

The Chevremont and Fredericq reaction is negative in the samples taken on the eighth, fourteenth, and twenty-fourth days.

CONCLUSION

The basal vaginal layer is characterized (1) by its richness in nucleic acids (DNA and RNA), in alkaline phosphatases and in potassium, and (2) by the absence of glycogen, polysaccharides and sulphydrilic protides. It does not seem to be affected by the cyclic modifications.

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In cytochemical studies observation may not necessarily lead to interpretation. The staining procedures are sensitive to slight variations in technique and although a strict discipline of uniformity is maintained a range of error is usually considered before significant interpretations are made. In addition, the interpretation of findings is impaired by the fact that the affinity for a special stain may not be specific.

In view of the fact that the morphology of the normal and atypical cells of cervical origin is relatively better understood than that of cells from other sites, the cytochemical studies in this area were deferred and the data presented at this time, therefore, is not complete.

When vaginal smears are stained by the Feulgen method, the parabasal cells appear to have a low relative DNA content as determined by photometric means. The nuclei appear to have a diameter of about 12 micra. In comparative studies parabasal cells with dyskaryosis revealed a greater amount of DNA than normal parabasal cells. Nuclear diameters appeared to be 3 micra or more, larger than that of normal parabasal cells.

PAS staining of vaginal smears revealed that parabasal cells are PAS negative, which indicates lack of glycogen. The absence of PAS staining of the parabasal cells was also noted when diastase was omitted.

The relative DNA content of intermediate cells, determined by photometric means of Feulgen stained smears, appeared to be within the range of parabasal cells, although in some cases an insignificant increase was found which remains unexplained at this stage. More marked differences, however, could be determined between normal intermediate cells and intermediate cells with dyskaryosis.

By utilization of PAS staining the nuclei of intermediate cells appear negative after diastase. It is worthy of mention at this point that a type of intermediate cell may be found which presents characteristic features, which consists of indistinct cytoplasmic borders of closely grouped cells. The cytoplasm is usually elongated and the grouping of closely adherent cells appears usually over a narrow longitudinal area. This is usually associated with varying degrees of estrogen deficiency. The endocervical smears in such cases contain parabasal cells. The PAS staining of these cells appears to be more homogenous than in other cell types which are PAS positive. Following diastase, however, no PAS can be demonstrated in these cells. It is interesting to add, and perhaps significant that this type of cell has not been encountered in cases of cervical carcinoma.

DISCUSSION

JEAN A. de BRUX, Paris, France:

The article by Nieburgs is extremely interesting.

It seems to me normal that the atrophic basal cells encountered in the vagina have a lower DNA content than do the basal cells taken from a normal epithelium (either by scraping or by biopsy).

But it is interesting to me that the DNA level of the dyskaryotic parabasal cells is much higher. I believe that this is because these cells are always very hyperplastic in the beginning because of their embryonic potentiality, since they arise from a metaplasia of replacement cells, either differentiated (basal cells) or undifferentiated (reserve cells). This would explain perfectly the difference between the normal intermediate cell and the dyskaryotic intermediate cell.

However, I did not understand very well the eliptic Nieburgs' thought, discussed in his last paragraph, concerning the PAS of grouped intermediate cells, associated with varying degrees of estrogen deficiency and with endocervical smears containing parabasal cells.

PRABHAKER N. SHAH and MRUDULA J. DAVE, Bombay, India:

Our observations are concerned with the PAS staining of basal and parabasal cells in the absence of obvious vaginitis or cervical erosion.

It is known that the presence of these cells in large numbers (40-100 %) indicates marked estrogen deficiency and that these cells do not contain glycogen. Recently, however, we had two classic cases of congenital adrenal virilism showing 82 and 90% of these cells in their vaginal smears before treatment. Of these cells, 52 and 71%, respectively, showed PAS positive, diastase soluble material. On cortisone therapy

there was gradual reduction in the number of cells without disappearance of glycogen. We feel that the cytochemical changes observed in such virilized patients or even some of the hirsute patients of unknown etiology are but the end results of the interaction of varied quantitative combinations of androgen and estrogen. The presence of estrogen in sufficient quantities in such cases, as measured by chemical methods, has been established by many investigators. It, therefore, appears that the antagonism between estrogen and androgen might be at the local level viz., vaginal mucosa. This characteristic pattern of circulating androgen, however, does not pinpoint the source - ovarian, adrenal or exogenous - of its production. In any case, it seems whorthwhile to pursue and develop finer cytochemical methods which would detect the presence of biologically active sex hormones - acting singly or in combination. Such techniques would certainly have obvious advantages over the chemical measurement of urinary metabolites of the sex hormones.

NO CLOSING REMARKS

COMMENTS ARE INVITED
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THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

HISTO- AND CYTOCHEMISTRY OF INTERMEDIATE CELLS

CONSTANTIN HEROVICI
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Nucleic Acids

At the level of the intermediate vaginal cells, a moderate, rather slight, quantity of DNA was noted, especially on the periphery of the nucleus. Clearly visible at all levels of this layer, our stain, a green methyl pyronine orange G, tints the cytoplasm of the cells orange, which denotes a very low concentration of ribonucleic acid in the cytoplasm. We have not noticed any cyclic modifications of the nucleic acids at the level of this layer.

Glycogen

The intermediate cells are richer in glycogen than any other vaginal cells, with definite localization and fine granulation, placed like intracellular crescents. This localization is due to alcoholic fixation. The premenstrual scrapings show very clearly an augmentation of glycogen in the intermediate level; the crescents become larger.

It should be noted that in case of infection the concentration of glycogen does not seem to be influential, unless in a sense of augmentation, for in the second localization, besides the crescents already described, characteristics of Trichomonas infections appear at the level of pre-nuclear halos.

PAS

The MacManus reaction is calculated absolutely with Best's carmine and shows that the polysaccharides follow the localization and evolution of the glycogen. In addition at the intermediate level this reaction shows an intercellular polysaccharide along cellular contours.

Alkaline Phosphatases

The localization of phosphomonoesterases I at the intermediate level, evidenced by the Gomori technique with sodium glycerophosphate, are nuclear and especially nucleolar and much less cytoplasmic. The vaginal scraping of the 14th day is the richest in alkaline phosphates in the intermediate layer.

At the level of histological cuttings of vaginal biopsies, the alkaline phosphatic activity in the intermediate layer is much less than that of the basal layer.

Potassium

The Marza technique is particularly positive at the level of the nucleoli and at the periphery of the nucleus. It seems here that the importance of the deposit of potassium coincides with the concentration of nucleic acids, that is to say, with the DNA for the nuclear periphery and with the RNA for the nucleoli.

As with the acid nucleo-proteins acids, we have not found any important concentration of cytoplasmic potassium nor modifications during the genital cycle.

Sulfhydryllic Protides

The Chevremont and Fredericqs reaction is slightly positive (very pale blue) in the intermediate layer of the vagina, studied through scrapings and vaginal biopsies. The vaginal scrapings of the fourteenth day are more colored than those of the eighth or twenty-fourth day of the cycle. This seems to confirm the work of Asscher and Turner (1955) and that of Verne, Gabe, and Schramm (1956), who found a clear augmentation at the time of the estrus of mice or after the injection of estrogen.

CONCLUSION

Therefore we see that if the nucleic acids and the potassium of the intermediate layer of the vagina do not seem to participate in the cyclic evolution of this mucosa, the alkaline phosphates and the sulfhydryllic protides do: the first two in the inverse sense and the last two proportionally to the estrogenic stimulation. For technical reasons we do not believe these facts precise enough to permit the evaluation of this stimulation.

DISCUSSION

PIERRE J. HAOUR, Lyon, Rhone, France:

This extensive and very interesting histochemical study of Herovici supplies valuable information about the chemical components of the vaginal layer, and about their variations. Our own experience is limited to desoxyribonucleic acid (DNA) in exfoliated cells.

DNA was measured by cytophotometry according to the technique described elsewhere (2). The DNA value of the intermediate cell was found by this technique to be twice the value of the human spermatozoid (2N). In inflammatory and dyskaryotic states this value may increase to 6N. Over this limit, the DNA dosage is in most of the cases correlated with cancer.

We agree with Herovici's findings concerning the absence of any cyclic variations of the DNA content of intermediate vaginal cells during the length of the menstrual cycle. However, we have noticed lower values after estrogenic treatment, but this experience is limited to a few cases and perhaps Herovici can give us his opinion about the action of estrogens.

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CLOSING REMARKS

CONSTANTIN HEROVICI:

In patients with mammary carcinoma, in whom the lack of any estrogenic activity resulted in estrogenic therapy and in whom I performed vaginal smears in order to check the therapeutic effect, I have never found change in the DNA-content in the intermediate layer of the vaginal epithelium.

I have to add that this observation has been made on a series of 100 patients who did not receive any additional treatment by irradiation. Nonetheless, it should be taken into account that at the time the paper was prepared, i.e. before July, 1959, I was not in possession of the histophotometer. All of my findings are based upon the Feulgen and Methyl green-pyronin-orange G technique, obtained with the normal microscope and therefore are less precise than the ones of Haour, who employed quantitative techniques. These changes probably are always minimal since they escape the normal microscopical observation.

HISTO- AND CYTOCHEMISTRY OF SUPERFICIAL CELLS AND ANUCLEATED SQUAMOUS CELLS

CONSTANTIN HEROVICI
Villejuif, Seine, France

Nucleic acids

The superficial layer of the vagina, characterized by the presence of pyknotic nuclei, is naturally very rich in DNA. Often neither cytoplasmic nor nuclear RNA network is found. We have not found any modification in the nucleic acids.

Glycogen

Glycogen is present in the cytoplasm of the superficial layer, more abundant around the pre-pyknotic cells. The distribution of the glucide is less clear than in the intermediate cells, but these aspects are more often seen in crescent shapes due to the fixation by alcohol (Vokaer).

During the cycle, modifications are noted in the quantity of glycogen, whose maximum quantity seems to be deposited in the pre- and post-menstrual periods, and minimum towards ovulation, but a quantitive appreciation of the estrinic activity based on this criterion seems of little value to us. On the contrary, we have found that the superficial cells always charged with glycogen in the case of elderly women who have an atrophic colpocytological formula, and who are under estrinic treatment. Another point, which underlines the influence of estrin on the metabolism glucidic, is the appearance of cells charged with glycogen in prostatic cancers treated by estrin. Therefore, it seems that between natural estrin and therapeutic estrin there is a paradoxical difference as to their influence on the cellular glucidic metabolism.

Polysaccharides

The reaction of PAS is very evident in these cells and seems to note a slight difference with Best's Red, for the most superficial layers are also the most PAS positive. The same cyclic modifications already mentioned are found with the MacManus technique.

Alkaline Phosphatases

Alkaline phosphatases are found in nuclear and cytoplasmic localization throughout the whole area of the superficial layer, and its maximum seems to be placed at the time of ovulation. One also has the impression that the curve of the alkaline phosphatase is symetrically opposed to those of the glycide metabolism.

One should note that we have been able to see the total disappearance of alkaline phosphatase under the effect of a treatment of restraining the folliculine, for example after the administration of testosterone.

Potassium

Potassium is localized in the cells of this layer on the same level as the nucleus, and to a lesser degree in the cytoplasm. We have not noted any cyclic variations of the potassium salt.

Sulfhydrillic Protides

Since the works of Giroud and Bulliard (4), Aescher and Turner (5), and Verne, Gabe, and Schramm (6) we know that keratinization is shown by the augmentation of the intensity of the reaction of the sulfhydrillic groups, in the malpighian epithelium and that modifications are found in the SH groups in mice during estrus and under estrin therapy.

Indeed with the Chevremont and Fredericqs reaction, the superficial cells of vaginal smears and vaginal biopsies are intensely colored blue, and at the moment of ovulation (smears of the fourteenth day) the maximum results are obtained from this reaction. At this time as we are going to see, even the intermediate cells are SH positive.

Conclusion

The superficial layer of the vagina as demonstrated by the cytological modifications of Papanicolaou study does take part in rhythmic cycle from estrogenic stimulation by its constituents demonstrated, such as: polysaccharide, glycogen, phosphatase alkaline and sulfhydrillic

protides. On the other hand their capital of nucleic acids and potassium does not seem to be influenced by the hormonal cycle.

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HISTOCHEMICAL STUDY OF CYTOPLASMIC GRANULATIONS

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The study of cytoplasmic granulations gave somewhat poor results.

We found, at the level of the basal layer, very fine pyroninophilic granulations which were identified as consisting of ribonucleic acid. Very rarely did we find melanin granules in this same layer shown by the Fontana stain. The most striking granulations were found at the level of the granular stratum of the Malpighian layer. Since the Chevremont and Fredericq reaction was intensely positive at this level, it seemed evident that the keratohyaline was particularly rich in sulfhydryl groups. In the vaginal smears we found an abundance of superficial cells rich in SH positive granules, especially in cases of infected uterine cervix.

TERMINOLOGY OF CYTOLOGICAL SMEARS IN REGARD TO ESTROGEN EFFECT

EMMERICH von HAAM
Columbus, Ohio, U.S.A.

Three methods are available to express the estrogenic effect demonstrable in cytological smears from the vagina or ectocervix: the Karyopyknotic Index, the Eosinophilic Index, and a verbal interpretation of the cytological picture. In a recent questionnaire put before 22 clinicians with regard to hormonal cytological reports, the majority preferred a comparative clinico-cytological evaluation either with or without the Karyopyknotic or Eosinophilic Indices, while only a few clinicians were willing to accept the Karyopyknotic Index or Eosinophilic Index per se as sole expression of hormonal evaluation (Acta Cytologica 2: 153, 1958). In the opinion of experienced cytologists, such as Pundel, the Eosinophilic and Karyopyknotic Indices are the most valuable qualitative and quantitative criteria of estrogenic effects. However, he admitted that there are some cases in which one has to evaluate the smear by other criteria (1). Wachtel (2) warns against relying too strongly upon the Eosinophilic Index since infections, chemical agents used in douches, cohabitation, etc. may materially influence the staining properties of the cells. Korte (3) found in comparative studies that cellular and nuclear measurements are inferior as compared with the visual optical interpretation by the experienced cytologist.

From the time we started to examine vaginal smears it has been our custom to evaluate the estrogenic effect demonstrated in the cytological picture and to report it to the referring physician. We are in full agreement with Gaudefroy (4) that carefully standardized technique is one of the most important requirements for the reliable estimation of the estrogen status of the woman by cytological criteria. Wied (5) recommends the middle third of the posterior or lateral wall of the vagina as the site of preference for the vaginal smear, which would mean in most cases that an additional smear must be examined by the already busy cytotechnologist. We have found that smears taken from the vaginal pool are usually satisfactory for the correct hormonal evaluation of the patient. It is very important that the age of the patient is always noted on the referral slip, as well as the date of last menstruation. Smears stained by the usual Papanicolaou method are used and the karyopyknotic index is determined by counting 100 single cells and recognizing pyknotic nuclei by size and staining property without micrometric measurements. Eosinophilic indices are used only in the hormonal evaluation of pregnancy smears. In most cases we prefer a visual optical interpretation of the estrogenic effect, which we express in degrees from zero to 3 plus.

A smear with a zero estrogen effect usually is composed predominantly of basal and parabasal cells with only very few eosinophilic superficial cells present. The staining reaction is pale cyanophilia, and the picture compares well with Fig. 1 of Korte's paper (3). The Karyopyknotic Index varies from 0 to 10 %. Smears of this type are found in advanced menopause, normally in women 60 years of age or older. It represents what we like to call an atrophic menopausal smear.

In a smear classified as "estrus 1 plus" parabasal and intermediate cells are usually predominant. The staining is still predominantly cyanophilic, and the Karyopyknotic Index varies between 10 and 40%. Smears of this type are commonly found in women in early menopause, in certain postpartum conditions, and in children of prepuberal age.

In a smear classified as "estrus 2 plus" almost the entire cell population is represented by intermediate and superficial cells. The Karyopyknotic Index varies between 40 and 80%, and a strong luteinizing effect is often evident. Smears of this type are found in normal women before, during and immediately after menstruation, in pregnant women, and in women with oligomenorrhea. They often persist for a considerable period after the onset of menopause. In smears interpreted as "estrus 3 plus" the overwhelming cell picture is that of eosinophilic superficial cells with a Karyopyknotic Index of over 80% and an Eosinophilic Index of at least 50%. Smears of this type are found typically in healthy women during the second and third weeks of the menstrual cycle. This type of smear is considered abnormal during menopause.

If the hormonal status of the woman is

normal with reference to her age and menstrual history, no further comments are made. However, if we consider the vaginal hormone evaluation as abnormal, it is emphasized in a note written by the pathologist at the end of the report with appropriate recommendations. This may include the recommendation of hormonal therapy of infectious processes, or of further examination of the patient to rule out ovarian tumors. We have found this terminology of the estrogenic effect in vaginal smears very satisfactory since it is easy to teach, and seldom do different cytotechnologists in our laboratory vary in their estimation of the estrus of the same vaginal smear. The clinician is very grateful for this service, and the estimation of the estrus of a patient from the vaginal smear has completely replaced the more time-consuming and expensive determination of estrogenic hormone levels in the blood and urine, which was previously performed in the biochemical division of our laboratory.

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The evaluation of vaginal smears for the determination of the degree of estrogenic stimulation may be carried out and reported either by description of cells found or in terms of the degree of estrogen effect. The latter method requires extensive knowledge of vaginal physiology and endocrine gynecology. Conversely, the clinician may have difficulties in evaluating a report which is limited to the description of cells or Karyopyknotic Index.

A low Karyopyknotic Index in the early proliferative phase reflects normal estrogen activity. The same ratio of karyopyknotic cells in the late proliferative phase would be indicative of a certain degree of estrogen deficiency. On the other hand, a high karyopyknotic index is not necessarily an indication of adequate or increased estrogen activity. While this is normally found in the late proliferative phase, its presence in the second half of the menstrual cycle is suggestive of non-ovulation. In addition, it appears that the vaginal smear alone is not sufficient and may often be misleading in the interpretation of estrogenic activity. In the presence of infection, it is not necessarily a reflection of the true state of hormone levels. Not infrequently a vaginal smear with a high karyopyknotic index, may be associated with a cervical smear which contains predominantly cells of the intermediate epithelial layer. Since the effect of estrogenic stimulation on the vaginal epithelium does not depend on the degree of estrogen activity alone, but also on the duration of estrogenic stimulation, a high karyopyknotic index may be found when decreased estrogen activity has prevailed over an extended period. Since the squamous epithelium near the columnar junction apparently does not respond to estrogenic stimulation as readily as the vaginal epithelium, the lack of karyopyknosis at the cervical site in such cases often indicates the true state of estrogen activity.

In the postmenopausal age, the cessation of ovarian function is associated with vaginal smears of varying types ranging from those with increased karyopyknosis to smears with persistent marked cytolysis to smears with intermediate or parabasal cells. In the older woman (10 years or longer postmenopausal) the finding of predominantly parabasal cells in the vaginal smear is rare. Not only may there be intermediate or superficial, cyanophilic cells, but the presence of a certain percentage of karyopyknotic cells is not a rare finding.

The vaginal terminology of decreased or increased estrogen activity based on the Karyopyknotic Index is therefore not entirely adequate unless the evaluation is made with due correlation to the prevailing circumstances. In pregnancy, a high Karyopyknotic Index is not interpreted as an indication of increased estrogenic stimulation, but as a result of decreased progesterone activity.

It is therefore difficult to suggest a uniform terminology for the reporting of the estrogen effect, and it is my opinion that the creation of a standard terminology for the effect of estrogen activity may not be of great advantage. A thorough knowledge of the vaginal physiology in relation to hormonal activity by either the gynecologist or cytologist may present a better alternative.

ABRAHAM E. RAKOFF

Philadelphia, Pennsylvania, U.S.A.

An acceptable terminology must (1) accurately describe the effect of varying amounts of estrogen alone on the vaginal epithelium, from none to the largest amount to which the epithelium will respond; (2) the terminology should be related to an available standard, based on adequate objective description of the cellular contents and (3) should be referrable to various physiologic states of estrogenic activity, such as may occur in children, certain phases of the ovarian cycle during the reproductive years, or the post-menopausal years.

From the scientific standpoint, the objective description of the cellular contents with as many quantitative measurements (percentages of cell types, Eosinophilic Index, Karyopyknotic Index) is unquestionably the most accurate. For usual clinical purposes the extensive descriptions can be replaced by a number of approximate "categories" into which the whole spectrum of estrogenic effect can be subdivided. The cytologist can then list the appropriate category on his report and can also indicate if he wishes, or if he is so requested, as to whether or not this is normal, for the clinical facts presented, such as age, and phase of the menstrual cycle; or the clinician can make this interpretation himself on the basis of his intimate knowledge of the patient's clinical status. It is

obvious that there are many smears which cannot be categorized in this simple fashion because of atypical findings resulting from the presence of hormones other than estrogen, such as progesterone or increased amounts of androgen, or the presence of inflammatory reactions. Here, cellular descriptions are essential, and accurate interpretation is possible only in the light of the full clinical findings. It is likewise apparent that for certain purposes the listing of an approximate category is not sufficient, as in the interpretation of the time of ovulation, in which there are more subtle changes occurring from day to day, and which vary with the individual patient.

For purposes of discussion I would like to present six categories of estrogen effect into which the broad spectrum may be subdivided and which occur with sufficient frequency in normal physiologic states and in various endocrinopathies to be clinically useful. In the accompanying Table they are listed from "0" to "5," in order of increasing estrogen effect, without the presence of other interfering hormones or inflammatory reactions. These cytologic patterns are correlated with the histologic type of vaginal epithelium, the urinary excretion level of estrogen most commonly found in association with the particular cytologic pattern, and those normal physiologic states in which this type of estrogen effect is found. It will be noted that in this classification, no attempt is made to include the corpus luteum phase of the cycle or pregnancy, since these present special problems of their own.

SUGGESTED CLASSIFICATION OF ESTROGEN EFFECT

Estrogen Effect	Cellular Description	Histologic Type	Approximate Urinary Estrogen Excretion m.u./ 24 hours *	Characteristic Physiologic State
"0"	Basal and parabasal cells only	Markedly atrophic	Less than 6	Early childhood, some postmenopausal women
"1"	Parabasal & intermediate cells	Atrophic; Very little proliferation	6-22	Childhood, postmenopausal
"2"	Chiefly intermediate, some parabasal & cyanophilic superficial cells	Intermediate Proliferation	22-24	Late childhood, postmenopausal
"3"	Chiefly superficial cyanophilic cells, low E.I. & K.I.,** some intermediate cells present	Moderate proliferation	44 to 66	Early follicular phase
"4"	Chiefly superficial cells with a moderately high E.I. & K.I.	Highly proliferated	66 to 132	Mid-follicular to pre-ovulatory phase
"5"	Practically all superficial cells with very high E.I. & K.I.	Highly proliferated	More than 132***	Pre-ovulatory and ovulatory phase

* Allen-Doisey assay, after acid hydrolysis and CCl_4 extraction
** The precise Eosinophilic and Karyopyknotic Index may be included if desired.
*** The vaginal smear response usually reaches a peak at excretion levels of 200 m.u.

DISCUSSION

IRENE BERNARD and RENEE CATOR, Bordeaux, France:

In the matter of determining estrogenic activity, we have noticed that on the great majority of curves, the Karyopyknotic and Eosinophilic Indices are almost parallel, the Eosinophilic Index being generally inferior by 5 to 10% to the Karyopyknotic index. From this we believe, like many other authors, that one needs to refer simply to the reading of the Eosinophilic Index in order to have the reading of the original smears. This method offers the advantage of simplifying the work in the laboratory and especially of avoiding the cause of errors due to a false interpretation in the deduction of pyknotic cells, which is not always easy because of the presence of cells in various stages of pyknosis.

It is understood that for the exactitude of such an estimate, all possible causes of errors must be eliminated. In this, we agree with de Brux. In some cases, however, there is a notable and constant disparity between the two Indices. Balans (Bordeaux) has pointed out that in these cases, the excretion of 17-ketosteroids was high.

The classification of Rakoff is tempting, but we are of the opinion that there is a risk of giving the impression of a more precise hormonal evaluation than the smears can, in fact, provide.

Like Wied (1) we advocate a simplified estimation based on the fact that there are really only two types of characteristic smears:

1. The atrophic smear, in which there is a predominance of basal cells.

2. The smear at the time of ovulation, presenting mainly superficial cells, eosinophilic, with a pyknotic nuclei. It is the estrogenic smear that we are looking for, particularly in normal women in full genital activity.

The examination of smears in other periods of the cycle is of lesser interest, since the smears are not characteristic (except the postovulatory ones). The abundance of intermediate cells indicates an undefined state of proliferation which can be brought about by hormones other than estrogenic substances: progestational agents and androgenic substances, or by the combined action of these hormones with the follicular hormone. One must remain very cautious about their hormonal interpretation.

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CLARICE do AMARAL FERREIRA, Rio de Janeiro, Brazil:

We can see from the main speakers the difficulty in stating a universal terminology. Each one proposes a different classification for vaginal estrogen effects. This is due to the fact that the smear we analyze is a result of the action of certain quantities of different hormones on the mucosa plus the reaction of this mucosa (depending upon many factors, including local inflammatory processes), depending upon the technical methods used and personal interpretations. This is why I think that for hormonal purposes all gynecologists should know how to evaluate a smear and to do it in accordance with other tests and with the entire clinical picture in mind.

MARCEL GAUDEFROY, Lille, Nord, France:

I am very astonished by the pessimism of Nieburgs. Perhaps his distrust for the value of quantitative interpretation of vaginal smears for estrogen effect is in connection with a technique which is not carefully standardized. Last year I saw many smears at Montreal, the Mayo Clinic and in New York, which were very good for cancer detection (with the use of the Papanicolaou stain or hematoxylin-eosin), but not satisfactory in my opinion for reliable hormonal evaluation, because the very great variations of cytoplasmic shade and pseudo-impossibility to correctly determine the Eosinophilic Index.

On the other hand, I agree with von Haam and Rakoff's trials to establish standardized methods of evaluation of the cycle of patients in correlation with clinical aspects. However, I use another terminology in interpreting estrogenic vaginal effects:

1. In menstruating women, the Eosinophilic and Karyopyknotic Indices (E.I. and K.I.) are compared with the normal percentages of E.I. and K.I. established by Pundel and others in a graph from the beginning to the end of the cycle, and if the E.I. and K.I. are higher or lower than normal in the whole cycle or at certain days only, I speak of hyper- or hypoestrogenism of the total cycle or of a certain period of the menstrual cycle.

2. In non-menstruating women, before puberty, or amenorrheic, menopausal or castrated women, I use a notation similar to von Haam's, with zero to three plus and the same colpocytological criteria.

In difficult cases, it seems to me very useful to give a complete description of the cytological pattern and adequate recommendations, and with this point of view, I quite agree with the statements of von Haam.

WOLFGANG KORTE, Bonn, Germany:

If the cytological diagnosis of functional conditions and functional processes shall yield useful information for the practitioner, the clinically trained and morphologically experienced cytologist has to submit clear and comprehensive reports. I fully agree with the opinion of von Haam. Carefully trained cytotechnologists can determine the Karyopyknotic and Eosinophilic Indices. Only the cytologically experienced physician is able to interpret the inter-relationships between morphologically demonstrable features and endocrinological conditions. Seldom does only one single factor have to be determined and interpreted in an individual smear. Experience is a question of acquired knowledge and time. The numerically established laboratory result (such as indices) will surely be necessary to com-

plete the not clearly evident optical findings. If one wants to scientifically determine the effect of estrogens, one should consider the appearance of the entire smear, the Karyopyknotic and Eosinophilic Indices and chemical estrogen determinations in blood and urine.

Every scheme has disadvantages and sources of errors. The classification of von Haam seems to be fully sufficient for practical purposes. I only have a formal objection: I should not say "estrus 0, 1, 2, 3" but according to Rakoff "estrogen effect." However, I would otherwise retain the classification of von Haam. Nieburg's reference to the cervix seems to me very valid. The cytological interpretation of cervical epithelium, however, is essentially more difficult. However, physico-chemical methods permit useful conclusions (such as the so-called fern-test).

HÉCTOR MUNGUÍA M. and ESTHER FRANCO G., Mexico, D.F., Mexico.

We agree with von Haam, Rakoff and Nieburgs that a visual interpretation, together with the percentage of cellular types and the Karyopyknotic Index (K.I.) and the Eosinophilic Index (E.I.) is the safer criterion for evaluation of the estrogenic effect.

In each case these findings should be compared with the clinical data (age, menstrual history, etc.) in order to decide if the estrogenic activity is "normal" or "abnormal."

By studying vaginal cycles of normal women, we have found K.I. and E.I. curves similar to the ones found by Pundel (1) and others. We believe,as does Gaudefroy (2), that these curves show, at the moment of ovulation, the index of the most important physiologic estrogenic effect and also the minimum physiologic index of the vaginal cycle. Beginning with those indices as the normal patterns, we use four grades of estrogenic activity:

Grade 0: No estrogenic activity (only basal and parabasal cells). Epithelial atrophy.
Grade I: Hypoestrinism. It is divided into three sub-grades: accentuated, average and slight. (These are related to the proportion of basal and intermediate cells and are always with low K.I. and E.I.)
Grade II: Evident estrogenic activity. It is also divided into three sub-grades: slight, average and good (predominancy of superficial, karyopyknotic cells and intermediate cells, with high E.I.). Here we see the physiologic changes of the normal menstrual cycle.
Grade III: Possible hyperestrinism (more than 90% of eosinophilic, karyopyknotic, superficial cells). In this grade, it is necessary to study more than one smear to establish this diagnosis.

In most of the cases we add one visual evaluation of the different cell types found, in order to obtain later comparative data. We also indicate to the clinician if the cytologic pattern is concordant or discordant with the received clinical information. Slight hypoestrinism will be concordant if the smear is obtained from menopausal women and discordant if obtained between the second or third week of the cycle.

Since there is a great variety of cytologic patterns in menopausal women, we only consider grade III and subgrade of good estrogenic activity of grade II as discordant.

We believe that the distinct terminologies suggested by the main speakers and other authors are good when utilized by experienced cytologists and, in addition, when understood by the clinicians; we believe that the statements of Pundel (3) about the criteria that the cytologist should use are more important than the "standardization" of terminology in order to avoid errors of interpretation.

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J. PAUL PUNDEL, Luxembourg, Luxembourg:

As shown elsewhere in this symposium, the Karyopyknotic and Eosinophilic Indices alone do not always reflect the true estrogen level in the female body, but only the end-result of the combined antagonistic or synergistic vaginal actions of all hormones able to influence the vaginal epithelium. Therefore, if one would classify the vaginal smears after the degree of cell maturation or after the Karyopyknotic or Eosinophilic Indices for the measurement of the estrogenic activities, one has to accept too many errors. Since the vaginal smears show only an end-result which is influenced not only by the estrogens, but also by progesterone and androgens, it appears that such a classification is acceptable only for conditions where any vaginal activity of progesterone or androgens can be excluded. This means that, in general, especially for hormonal evaluations of the menstrual cycle, pregnancy and postmenopause, we cannot rely upon rigid numerical smear classifications. I have pointed out in many papers that the best standard terminology for hormonal diagnosis, as suggested by the main speakers, is to relate the smears to available standards of the normal conditions, while exclusive numerical classifications should be reserved for research work or for testing hormonal substances in the castrated woman.

For the usual hormonal diagnosis by vaginal smears, the following steps have to be considered:

1. The side of the vagina from which the smear has been taken. Only smears from a lateral wall should be accepted for hormonal diagnosis.

2. For the evaluation of the stained smear, one has to first consider the general pattern (cell types present, type of shedding, single cells or clusters, flat or folded cells, cell alterations by infection or chemical agents, absence or presence of trichomonas, etc). This first general study is followed by the count of the Karyopyknotic and Eosinophilic Indices.

3. From these findings, the cytologist tries to make a general hormonal ev-

aluation, then he comes to the final diagnosis, where he has to make the practical conclusions needed by the clinician.

This final diagnosis is the result of the comparison of the hormonal evaluation of the examined smear with the clinical data. To illustrate it, I choose the following example: a smear showing a pure, but medium estrogen effect (isolated and flat superficial and intermediate cells, no clusters, only occasional folded cells, Karyopyknotic Index of 50, Eosinophilic Index of 35. Related to the different possible clinical data, it permits the following conclusions for diagnosis:

a. Smear taken at the 11th day of a menstrual cycle of 28 days: Normal estrogenic stimulation.

b. Smear taken at the 14th day of a 28 day cycle: Insufficient estrogenic stimulation for a normal 14th day.

c. Smear taken at the 23rd day of a 28 day cycle: Medium estrogenic stimulation. No progesterone effect. Anovulatory cycle probable, but for a precise diagnosis, a series of repeated smears is requested.

d. Smear taken at the 80th day of amenorrhea: Medium estrogenic stimuli corresponding to that of the 11th day of a normal cycle.

e. Smear taken from a woman 75 years old: Abnormally high estrogenic stimulation, if exogenous estrogens can be excluded, for a patient 25 years after the menopause.

f. Smear taken at the 5th month of pregnancy: Abnormal pregnancy smear, manifesting hormonal dysfunction.

I think that this way of reporting the cytological conclusions would be more useful for the clinician than either pure cytological descriptions or reports of numerical criteria which the clinician has to interpret in his own manner. But for this, he must have a rather good knowledge of cytology, which is not always the case.

CLOSING REMARKS

EMMERICH von HAAM:

The great variety in the discussion of this subject reflects the great differences in the opinions also expressed in the contributions by the above authors. I do believe that Bernard and Cator do not do full justice to the diagnostic possibilities of the vaginal smear by only recognizing the atrophic and estrogenic types of smear. On the other hand, Munguia and Esther Franco go too far by trying to differentiate eight different grades of estrogenic activity, each of which may be concordant or discordant with the clinical information. We are in hearty agreement with Ferriera and Pundel who, after putting down stringent rules for a standardized technique, present an interpretation of the vaginal smear in relation to numerical indices. Pundel's example is well illustrative of this fact and also emphasizes one thing often overlooked, that in order to give a clinically useful interpretation the cytopathologist must also be familiar with the significance of clinical data furnished by the referring physician. Korte's formal objection to my classification is gratefully accepted.

IS THE KARYOPYKNOTIC INDEX A MEASUREMENT OF ENDOGENOUS ESTROGENS?

GUILLERMO TERZANO
Buenos Aires, Argentina

From the basic papers of Papanicolaou and Shorr we have learned that the Karyopyknotic and Eosinophilic Indices in vaginal smears can be considered as a criteria for estrogenic activity. Many papers on the subject have confirmed the fact that the qualitative and quantitative changes in the appearance of the vaginal epithelium, as revealed by vaginal smears, are proportional to the amount of estrogenic activity in the body.

Percentages of superficial cells in vaginal smears throughout the life of a woman show striking cytological characteristics: a) transitory peak in the newborn, b) absence (atrophy) during childhood, c) increase as puberty approaches (development of the ovarian follicles prior to menarche), d) cyclic ascending and descending curves during the menstruating period, and e) decrease after menopause towards atrophy, which is considered as one of the signs of the female climacterium. There are, also, the remarkable changes after castration.

We have reliable evidence that karyopyknosis (superficial cyanophilic cells) is an indication of endogenous estrogen. The Karyopyknotic Index should be considered conclusive, however, if it coincides with an increase in the Eosinophilic Index, since we know that the Eosinophilic Index has a higher threshold level than the Karyopyknotic Index (in slides stained according to the Papanicolaou Technique).

In any case, vaginal smears taken with proper precautions may be used as a reliable qualitative test for the estrogens of the patient, being the percentages of the pyknotic cells. Also, the eosinophilic cells are a useful test for the increase decrease of estrogen levels when serial vaginal smears of the same patient are examined. They are also of value in assessing single vaginal smears.

Nevertheless, attention must be paid to possible causes of error. It is known that the degree of pyknosis and eosinophilia may be influenced or artificially raised by non-hormonal factors, such as friction due to pessaries or contraceptive diaphragms, inflammation, parasites, suppositories, vaginal douches of common use (iodine tincture, potassium permanganate, lactic acid, etc.), vitamins, radium therapy, etc., or by hormones other than estrogen.

We might mention here the low Karyopyknotic Index in smears of normal pregnant women.

The above facts do not mean that the Karyopyknotic Index is not an indication of endogenous estrogen. However, there is need for the greatest caution in regarding high or low percentages of superficial cells as synonymous with high or low estrogenic levels.

Summarizing: we consider that in assessing ovarian function, karyopyknosis and eosinophilia are a reliable qualitative assay of the patient's endogenous estrogen. Evaluation is based upon the percentages of cells and the pattern of correctly prepared smears.

ERICA WACHTEL
London, England, U.K.

The Karyopyknotic Index in vaginal smears is, in my opinion, a reliable yardstick of the action of unopposed estrogen. This can be demonstrated by the high Karyopyknotic Indices found in smears of women with estrogen producing tumors, and the steady rise of Karyopyknotic Index readings during the proliferative phase in normally ovulating women. However, as soon as the corpus luteum begins functioning and progesterone simultaneously acts upon the vaginal epithelium, the Karyopyknotic Index ceases to indicate the degree of estrogenization. For the same reason, during the course of normal pregnancy, the Karyopyknotic Index fails to indicate the presence of the physiologically increased estrogen production. A rise in Karyopyknotic Index readings during pregnancy must not be interpreted as an increase in estrogen, but as a disturbance in the estrogen/progesterone balance, i.e., a progesterone deficiency.

In summary, the Karyopyknotic Index alone is, therefore, a reliable indicator of endogenous estrogen production only in such cases where there is no interference from other sex hormones acting upon the vaginal epithelium.

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany

We are also of the opinion that the Karyopyknotic Index is a reliable measurement of estrogen activity, especially if the Eosinophilic Index and the indices of the various forms of cells are included in the judgement.

In regard to the assessment of the endogenous estrogenic activity, we agree with both authors that the Karyopyknotic Index is only conditionally useful. This is because, in the presence of various hormones simultaneously acting in the organism, the vaginal epithelium is able to reflect only the effect of the relatively predominating one.

Errors may occur by non-hormonal influences, such as inflammatory changes, infections of the vagina, local use of disinfectants or astringents.

Furthermore, it is of decisive importance which method is employed for determining the Karyopyknotic Index. As Pundel and Lichtfus have proved and as we were able to verify by our own tests, the values differ highly from one method to another. Only Karyopyknotic Indices determined by the same method can be compared with one another. Instead of obtaining this Index by measurement of the diameters of the nuclei (e.g., up to 5μ or 6μ), we believe that it is more physiological to find it by estimation of the pyknotic nuclei or by an evaluation using the phase-contrast microscope on stained preparations (3).

The diameters of the nuclei are, of course, objectively and exactly comparable measurements, which can be reproduced by all examiners. But this does not conform with the actual nuclear pyknosis, because some non-pyknotic nuclei are mistakenly included and occasionally there are larger, but still pyknotic, nuclei eliminated.

On the other hand, the count of nuclei judged pyknotic by normal microscopic observation have the disadvantage that certain subjective factors may influence the results. This can be mostly avoided by using the phase contrast method for determining the Karyopyknotic Index on stained smears. However, the same staining method always has to be used in order to obtain comparable values. As we have found out, the Karyopyknotic Index is essentially higher with the Shorr-hematoxylin stain than with the Papanicolaou stain.

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MARCEL GAUDEFROY, Lille, Nord, France:

I am quite in agreement with Terzano and Erica Wachtel. Like Terzano, I point out that the Eosinophilic and Karyopyknotic Indices are "approximative" criteria of estrogen activity. The amount of endogenous estrogens is revealed by colpocytology only "grosso mode," enough, however, for the clinical use and enough to take the place of the hormonal dosages. The hormonal assays are not safer from the point of view of the true quantity of produced estrogens. Erica Wachtel is right to say that the K.I. and E.I. are reliable indicators of endogenous estrogen production only in cases when there is no interference from other sex hormones acting upon the vaginal epithelium. However, in the luteal phase of menstrual cycle, increased K.I. and E.I. frequently occur and that increase indicates excessive predominance of estrogens if the folding and clumping of cells are well marked, and a relative insufficient progesterone level if the folded cells are decreased.

GILDARDO LEGORRETA, Mexico, D.F., Mexico:

I agree with Terzano and Erica Wachtel. Taking into account the whole hormonal pattern and associating the Karyopyknotic Index with the Eosinophilic Index, useful information can be drawn from the range and speed of changes in series of smears, if compared with cytologic patterns already known.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I agree completely with the main speakers. In general, the Karyopyknotic and the Eosinophilic Indices are closely related to the estrogenic activity, and as the eosinophilia can be produced by other non-hormonal factors, the Karyopyknotic Index is a rather specific criterion for the measurement of endogenous and exogenous estrogens. But both Indices can be accepted as a true indicator of the estrogenic activity only if one can exclude artificial factors and other hormonal activities. The same conclusion is equally true for the endometrium where atrophy does not always mean a complete absence of estrogen secretion. Therefore, the Karyopyknotic Index corresponds to the real estrogenic activity in the body only if the estrogen effect on the vaginal epithelium is not influenced by other hormones. This happens in normal conditions only during the first half of the cycle and during childhood. In the other phases of the female life, or in pathological conditions, the vaginal epithelium is under the influence of associated hormones like progesterone and androgens. In these cases, the Karyopyknotic Index as well as the Eosinophilic Index will not show the true estrogenic level, but only the end-result of all hormonal actions working in the vaginal epithelium. The best example is given by the pregnancy smear as shown by Erica Wachtel, where both Indices are under 5 after the third month, at a moment where the estrogen production is very high. But the estrogens are blocked in their pyknotic effect by the high

amount of progesterone secreted at the same moment. During this time, a rise of the Karyopyknotic Index in general does not mean a rise of the estrogen secretion, but only a lowering of the progesterone activity, and if the latter shows a marked regression, the Karyopyknotic Index can rise even if the estrogens follow this regression, but remain the predominant hormone acting on the vaginal epithelium. Therefore, the measurement of the endogenous estrogens has to be based not only upon the Karyopyknotic Index, but also upon the general pattern of the smear with all the cytological details. This evaluation, furthermore, has to be related to the clinical data.

LUIS MONTALVO RUIZ, Madrid, Spain:

We agree with the authors that the Karyopyknotic Index is a good and safe evaluation of the activity of endogenous estrogens. We have confirmed this by making on the same day an endometrial biopsy and a cytological smear. There was close correlation between the two methods. In our paper published in Acta Cytologica (2:623, 1958) this was also shown very clearly. The only failures were attributed to vaginal infection or lack of receptivity of the vagina to estrogens (one patient had received intravaginal radium).

Other hormones with a simultaneous action on the vagina (as the progesterone and androgens) can also modify the true value of the Karyopyknotic Index.

In pregnancy the Karyopyknotic Index is not a reliable key of the amount of endogenous estrogens because, besides the increase in progesterone levels, there is one hyperproliferation in the intermediate layers of vaginal epithelium with desquamation of sheets of cells. This prevents their maturation and their transformation to superficial karyopyknotic cells because they are shed before this could possibly occur.

HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D.F., Mexico:

We agree completely with Terzano that the Karyopyknotic Index (K.I.) is a real qualitative evidence of the endogenous estrogens if the smears are taken and processed with all precautions and the non-hormonal factors that influence this index (infections, irritations, douches) are discarded.

If, together with the high K.I., we observe an increase of the Eosinophilic Index (E.I.) a definitive criterion is obtained as to the existence of high levels of endogenous estrogens.

Concerning the statements of Erica Wachtel, we would like to add the following comments:

The K.I. does not necessarily mean a low level of estrogens, primarily during pregnancy. In the second half of the menstrual cycle, the lowering of the K.I. is explained not only by the presence of progesterone produced by the corpus luteum, but also by lowering of the level of the estrogenic hormone. It is out of the question that the experimental or therapeutic administration of progesterone and androgens lowers the K.I.; thus, we agree with Erica Wachtel that when the vaginal smear is submitted to the simultaneous action of several hormones, the K.I. loses its sensibility as a qualitative indication of the level of estrogenic activity.

During pregnancy the high estrogenic level is not evidenced by a high K.I. and the problem is to know if the vaginal epithelium does not respond to estrogens because 1) sensibility of the vagina is modified or 2) because its action is opposed by progesterone or 3) because the estrogens are transformed into progesterone and so do not act as such, in which case the K.I. would not lose its value as a measure of the "actuating" endogenous estrogens.

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CLOSING REMARKS

GUILLERMO TERZANO:

The comments seem to make it clear that karyopyknosis can be considered as a reliable qualitative assay of the patient's endogenous estrogens if evaluation has been made on actual pyknosis and if it is related to the clinical data.

IS THE PRESENCE OF EPITHELIAL ATROPHY A DEFINITE CRITERION FOR LACK OF ESTROGEN PRODUCTION?

ARTURO ANGEL ARRIGHI
Buenos Aires, Argentina

The presence of parabasal cells and the alteration of the normal vaginal flora characterize the atrophic cytological picture in vaginal smears. We use the classification of De Allende and Orias (1947) to determine different grades of cytological atrophy: 1 to 15% parabasal cells: light atrophy; 15 to 40% parabasal cells: moderate atrophy; 40 to 100% parabasal cells: advanced atrophy.

For a long time it has been known that this atrophic cytologic picture belongs to a similar picture in the vaginal mucosa; characterized by a decreased depth of the epithelium and the absence of the more superficial layers of the stratum spinosum. Similarly, it is known that these cytological and histological changes of atrophy are related to the lack of estrogenic hormonal stimulus. In 1936, Salmon and Franck, in their classification, related this cytological picture to profound lack of estrogen.

The atrophic smears are found in prepuberal girls, in old menopausal females, in the postpartum period and in amenorrheic patients with intense ovarian failure - known as the hypoestrogenic type. In all these conditions estrogenic lack has been demonstrated.

JEAN A. de BRUX
Paris, France

In general, the Karyopyknotic Index is the best test for the determination of estrogenic activity.

There are, however, some exceptions to this rule; the causes may be:

I. Exogenous or external:

A. Local chemotherapeutic treatment ulcerating the vaginal mucosa;
B. Local physiotherapeutic treatments;
C. Treatments with new steroid compounds having progestational action.

Wied and Davis have made a comparative study of the action of these substances:

17-alpha-hydroxyorogesterone
17-ethynl-19-nortestestosterone

noting the histological modifications of the vaginal cytology, the behavior of the cervical mucosa, and the basal body temperature. These substances, given in certain doses, have an atrophic action on the vaginal mucosa, although the estrogenic production remains normal or even elevated.

II. Endogenous or internal:

A. Ulcerations of the vaginal, and especially of the cervical, mucosa - resulting from inflammatory processes or from re-epidermization of a large cervical ectopy; however, the diagnosis of this is easily made.
B. The simple dysplasias of normal cellular morphology but exfoliating no pyknotic cells.
C. Strictly localized atrophy of the vaginal and cervical mucosa. Certain kraurosis in young women spread progressively from the labia as far as the cervix, whereas the endometrium responds normally to the ovarian stimulations and menstruation occurs regularly. This is a pathological situation difficult to explain, showing the important part played by the nervous system in the sensitivity of the receptors to the different hormones. The role of the metamesic segments of the nervous system should be investigated.

ERICA WACHTEL
London, England, U.K.

Epithelial atrophy in vaginal smears manifests itself by the absence of superficial squamous cells and the predominance of parabasal cells. The vaginal mucosa in women suffering from lack of estrogen is much thinner, owing to loss of superficial layers, than the mucosa of women with abundant estrogen. Exfoliation, therefore, occurs from the deeper layers. The same mechanism is, however, at work in women with inflammatory disease in the vagina and ectocervix. Here the thinning of the mucosa is not due to lack of hormone supply, but to the destruction of the superficial layers by micro-organisms or corrosive chemical substances. It may be easy to differentiate the "clean" endocrine atrophic pattern from the inflammatory pattern characterized by excess of leukocytes, histiocytes, micro-organisms and nuclear atypicalities. On the other hand, difficulties may and do arise in individual cases, e.g., it is extremely difficult, if not impossible, to decide whether the high percentage of basal cells is the result of lack of estrogen or due to the presence of infection in elderly women suffering from vaginitis. It is surprising how small the percentage of truly atrophic smears is in women long past the physiological cessation of menstruation or after surgical or radiological castration.

DISCUSSION

IRÈNE BERNARD and RENEE CATOR, Bordeaux, France:

We are in agreement with Pundel in recognizing that, in young women treated with a massive dose of irradiation, persistent vaginal atrophy is rare. After a transitory period, the vagina once more takes on its estrogenic aspect. The estimation of the residual function of the ovary in those cases where a total hysterectomy has not been performed is difficult to specify, and to differentiate from the estrogenic activity due to adrenal action.

A study of this discrimination should be made.

MARCEL GAUDEFROY, Lille, Nord, France:

I quite agree with the three main speakers. Particularly, Erica Wachtel, I would emphasize that it is often difficult to differentiate the true hormonal atrophy from the inflammatory pseudo-atrophy. This often occurs at the menopausal or premenopausal period in women having trichomoniasis and taking daily vaginal douches. Due to the frequent douches, the smears are clean, without leukocytes, microorganisms and trichomonads. The Eosinophilic and Karyopyknotic Indices are very low or nil and the cells are almost exclusively intermediate and parabasal. The cytologist, who does not know that vaginal douches were taken, evaluates the smears: subatrophic smear, hypohormonal state. Perhaps that woman is a premenopausal hyperfolliculinic one, verified by subsequent endometrial biopsy! A little cytological sign has permitted me to avoid this mistake in several cases: the nucleus of the intermediate and parabasal cells is not quite normal; it has naturally the shape and size of a parabasal or intermediate cell, but the nuclear limit is slightly irregular, finely indented (rumpled) with clear chromatin.

I believe that etiology explains some of the apparent vaginal non-receptivity.

PIERRE J. HAOUR, Lyon, Rhone, France:

Dr. de Brux gives a clear review of the circumstances in which basal cells are found in the smears, although it does not always correspond to a lack of estrogen production. Erica Wachtel rightly insists on the differences between atrophy due to estrogenic deficiency and an ulcerative process which is produced by inflammation. In both conditions basal cells are found in the smears, which sometimes may be erroneously interpreted as indicating lack of estrogenic stimulation. However, I believe that ulceration of the vaginal and cervical mucosa due to inflammation or local chemotherapeutic treatment must be considered separately since this subject deals primarily with epithelial atrophy.

In our opinion there are two types of atrophy: "extensive atrophy" and "localized atrophy" of the vaginal and cervical mucosa.

These conditions can be separated by colposcopy and microcolposcopy and by biopsy of the mucosa. Vascularization defects, which may be the cause of atrophy, are demonstrated very well by microcolposcopic observations which show vascular anomalies, with dilations of the vessels and blood stasis. The atrophic mucosa microcolposcopically appears reduced to a few layers of basal and parabasal cells and also cells of the intermediate and transitional type.

Generalized atrophy appears in a limited number of cases, as is pointed out by Erica Wachtel after menopause and castration and also in a low percentage of amenorrheas (mainly primary amenorrheas, cases with Turner's syndrome, and hypogonadotrophic cases). The smears are usually scanty and often autolytic. Basal, parabasal and a few intermediate cells are encountered in over 50% of the cases; karyopyknotic superficial cells are absent in this type of smear. Karyopyknosis is, however, frequently found in basal cells and parabasal cells, due to degenerative changes.

This condition is characteristic of lack of estrogen production. When this condition is associated with inflammation, as is pointed out by Erica Wachtel, vaginal smears are not easily differentiated from smears taken in ulcerative inflammation without estrogen deficiency.

Localized atrophy is represented by limited atrophic areas. It is mainly observed after hysterectomy (as described elsewhere in this Symposium) and after radium therapy. The atrophic zones are localized in the vaginal fornix or on the cervical mucosa. The same types of cells are encountered as in extensive atrophy,

but the smears also contain superficial karyopyknotic cells.

We believe that every time basal cells are encountered with superficial cells and especially with karyopyknotic eosinophilic cells, it indicates estrogen production.

In 60 postmenopausal patients who had hysterectomies and lymphadenectomies for cervical cancer, we found only 27% showing extensive atrophy (with basal and parabasal cells over 50% and no superficial cells).

The remaining 73% showed parabasal cells, but predominately intermediate cells with the presence of superficial karyopyknotic cells (with a Karyopyknotic Index varying from 0 to 30% in 32% of the cases and from 30 to 50% in 41% of the cases).

In conclusion; when basal or parabasal cells are found associated with pronounced inflammatory conditions, as mentioned by Erica Wachtel or when they are mixed with karyopyknotic superficial cells or with a higher percentage of intermediate cells, one has to be careful in interpreting the smear as atrophic (lack of estrogen production).

Appreciation of the Karyopyknotic Index of the urinary smear (urocytogram) and estrogen assays are used by us in these circumstances in an attempt to properly evaluate the hormonal conditions.

HERBERT JANISCH, Vienna, Austria:

The vaginal epithelium rather accurately reflects the status of ovarian function. The vaginal epithelium reacts especially well to estrogenic stimuli. Even dosages of estrogens which are below the threshold dosage on the endometrium will usually induce a slight estrogenic effect on the vaginal epithelium. Both Indices, the Karyopyknotic and Eosinophilic, react properly to estrogenic stimulation. In cases of estrogen deficiencies the loss of turgor of the cells is also of importance, in addition to the usual occurring regression of epithelial growth to the so-called atrophic menopausal pattern. One finds atrophic patterns in childhood, during the postpartum period, in menopause and after surgical and radiological castration, as well as in hypohormonal amennorrheas. I agree in this respect with Arrighi and Erica Wachtel. In other cases of estrogen deficiency one encounters a so-called mixed type which expresses apparently a subthreshold dosage on the vaginal epithelium. We understand under a "mixed cell type" a smear which contains intermediate and parabasal cells and sometimes a few superficial cells. The intermediate cells, however, closely resemble the parabasal cells due to loss of turgor. The high sensitivity of the vaginal epithelium to minute estrogenic dosages may be the reason for the relatively small percentage of completely atrophic smear types during menopause, as Erica Wachtel has reported. However, one may stress that smears resembling the atrophic cell type may also occur in cases where no estrogen deficiency is present. Those pseudo-atrophic types may be found in inflammatory reactions or chemical irritations of the vaginal epithelium. The purely morphological differentiation of these pseudo-atrophic types from the true atrophic types (which are due to estrogen deficiency) may pose considerable difficulties.

ALEXANDER MEISELS, Mexico, D.F., Mexico:

An atrophic smear, which contains over 90% basal and parabasal cells and does not show morphologic alterations of the cells, can be considered proof for lack of estrogen production. The only exception would be the very rare case of non-receptivity of the vaginal epithelium, when an atrophic smear can be obtained. However, the biopsy of the endometrium will prove adequate estrogen production. It is controversial whether this non-receptivity of the vagina exists at all, although it is a theoretical possibility.

When a smear contains basal or parabasal cells desquamated from cervical ulcerations, these cells usually show slight morphologic abnormalities (vacuolization of the cytoplasm, slight irregularities in the chromatin distribution), and furthermore, enough superficial cells will coexist in the smear to show that a certain level of estrogen production is present.

It is sometimes more difficult to answer the reverse question: To what extent are superficial cell smears a proof of adequate estrogen production? Infections, trichomonads, leukoplakias, prolapse, and many steroids (endogenous and exogenous) can stimulate the appearance of superficial cells in smears even in the absence of estrogens. Also, in these cases, the superficial cells show morphologic changes that point to non-estrogenic reasons for their presence.

HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D.F., Mexico:

We have observed that in the cytological picture of epithelial atrophy in postmenopausal women, children and pre-puberal adolescents, the estrogenic hormone is practically absent.

The problem posed by Erica Wachtel is a real one. The difficulty of determining, in adult women with severe vaginitis, whether or not the high percentage of parabasal cells seen in the smear corresponds with an absence of estrogenic stimulation or with the infection, is always present. Fortunately in the majority of the cases, the inflammation can be eliminated or improved and another smear gives a definitive conclusion to the case.

The atrophic smears produced by the administration of progesterone or androgens is another, completely different problem. To 4 patients with advanced mammary cancer and a smear of marked estrogenic activity, we administered 500 mg daily of one dihydrotestosterone compound and an atrophic smear was obtained in 3 cases. The biologic determinations of hormones in these 3 cases have demonstrated that the estrogens have diminished in all, but have not completely disappeared.

This evidence indicates clearly that the atrophic smears induced with antagonistic hormones do not indicate the absence of production of estrogens.

OTAKAR NYKLÍČEK, Náchod, Czechoslovakia:

In answering the question, "Is the presence of epithelial atrophy a definitive criterion of lack of estrogen production?" I agree with the conclusions of the main speakers, first of all with de Brux. The greatest difficulty for a cytologist is presented by a cytohormonal evaluation of parabasal cells with a simultaneous inflammatory process present in the vagina. The evaluation of fern-like phenomenon of cervical mucus and then the repetition of the smears after healing of the inflammatory process helps us in differentiation.

The question of the occurence of parabasal cells in smears taken postpartum is more complicated. In spite of a sudden strong depression of the level of estrogen, it is undisputable that here even prolactin plays a certain part.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

Vaginal atrophy and atrophic vaginal smears are in general related to a more or less complete lack of estrogen production, what we should call true atrophy. But parabasal and especially deep basal cells can also be seen in patients where a lack of estrogens can be excluded. These cells in the smear do not result from a true atrophy, but are the result of a local destruction of a rather normal epithelium, either by infection, chemical or mechanical trauma or due to particular modifications of the vaginal epithelium by physiotherapeutic treatment or treatment with steroid hormones. However, I agree with Erica Wachtel that most of these cases present no serious difficulties for differential diagnosis, especially in cases of local infection or irritation. As there exist practically no other cases of spontaneous vaginal atrophy not related to a lack of estrogens, there will be no serious practical difficulties for the cytologist or for the clinician, if the smear has been taken under the general rules for cytological examinations (smear taken under visual control and mention made on the laboratory form of previous treatments or existence of local disease). I agree with Erica Wachtel that the percentage of truly atrophic smears is very low, even in women long past the menopause or after castration. There remain the particular cases of kraurosis in young women as shown by de Brux. But I suppose that we have to deal here with an organic disease, like some dystrophies or dysplasias of the endometrium which also can present serious difficulties for the hormonal evaluation of an endometrial biopsy. Fortunately these cases are rare, but they show the need for the close relation of vaginal smears and endometrial biopsy in difficult cases and they should remind us that in cases with difficult vaginal smears which are taken from a visible local disease of the vagina one can also take a vaginal biopsy.

PRABHAKER N. SHAH and MRUDULA J. DAVE, Bombay, India:

It is generally recognized that basal and parabasal cells when present in considerable numbers (e.g., 40-100%) indicate marked estrogen deficiency (1). In addition, the Karyopyknotic Index is perhaps the most dependable criterion for assessing the level of circulating estrogen in an individual, if one were to exclude the external and internal factors which would upset this Index. However, there are some clinical situations where the Karyopyknotic Index may not correctly reflect the estrogenic activity. The case in point is where sufficient amount of androgen is alleged to be present pari passu with estrogen. We have found a low Karyopyknotic Index (varying from 1-26%) and different grades of cytological atrophy as suggested by de Allende and Orias in a number of cases (13 out of 26) having hirsutism of unknown etiology (2, 3). These basal and parabasal cells contain PAS positive, diastase soluble material indicating the presence of glycogen. These findings are intriguing since the occurrence of glycogen in basal or parabasal cells is paradoxical (in the absence of vaginitis or cervical erosion), if the low Karyopyknotic Index correctly reflects the estrogenic titer in these individuals. We have checked this observation in cases of congenital adrenal virilism where definite and higher titers of circulating androgen are known to be present. The reader is advised to refer to our discussion on "Cytochemistry of Basal and Parabasal Cells," in this issue.

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CLOSING REMARKS

JEAN A. de BRUX:

I believe Nyklicek's idea to look for the fern-like phenomenon of the cervical mucus in order to differentiate true atrophy from any inflammatory picture is very good. However, I am not absolutely sure parabasal cells in the postpartum period have a squamous cell origin. I believe many of them are decidual cells. I suggest that he make imprints of decidua and compare them with the cells found in the vagina in order to prove this fact.

ERICA WACHTEL:

It seems that, on the whole, we are all agreed on the value and the limitations of vaginal cytology in determining degrees of atrophy. Gaudefroy's observations on changes in vaginal smear patterns after repeated douching are very interesting and the point should be made, therefore, to ask every woman prior to taking the smear whether or not douching has been practised. I was also particularly interested in Haour's observation that 41% of his surgically treated cervical cancer patients had Karyopyknotic Indices between 30-50%. I wonder if he could let us know how many of these women were subsequently found to have residual malignant disease.

CELLULAR DEGENERATIVE CHANGES (CYTOLYSIS, AUTOLYSIS) AND ENDOGENOUS ESTROGENS

WERNER BICKENBACH and
HANS-JÜRGEN SOOST
Munich, Germany

We (2), like Wied and Christiansen (3), as well as Pundel and Ost (1), divide the degenerative cell changes, which occur in a large number of vaginal smears and which make exact hormonal diagnosis difficult or even impossible, into bacterial cytolysis and degenerative autolysis.

Bacterial Cytolysis is caused by Döderlein bacilli (Bacillus vaginalis) which require glycogen from the vaginal epithelium for the formation of lactic acid. This process is dependent on the presence of glycogen in the intermediate and in the superficial cells. Atrophic vaginal epithelium does not provide a suitable environment for the Döderlein bacilli and they cannot subsist. The glycogen in the eosinophilic (karyopyknotic) superficial cells is not, as a rule, available to the Döderlein bacilli due to the formation of prekeratin in their cell plasma.

Bacterial cytolysis is not a specific criterion of estrogenic activity. It can occur in the presence of any hormone which causes proliferation of the vaginal epithelium only to the lowest level of the intermediate cells: slight FH deficiency as well as good FH activity, both the FH and LH phase of the normal menstrual cycle, pregnancy, the influence of androgens and the action of several other steroid hormones. Mild degrees of cytolysis still renders impossible rough estimation of the hormonal status. Occasionally the presence of large numbers of free nuclei and intermediate cells with few superficial cells and marked FH activity, indicates such rapid dissolution of the actively proliferating vaginal epithelial cells that these latter cannot attain maturity.

The variability of the hormonal situation underlying the picture of bacterial cytolysis can be easily proved. When the Döderlein flora is temporarily destroyed by the insertion of an antibiotic into the vagina, the smears show all of the variations described above. In view of the expected side effects, it appears advisable to make use of this procedure only in exceptional cases.

Degenerative Autolysis is evident in three cell groups: parabasal and basal cells, endocervical cells, and malignant cells. Degenerative autolysis is independent of the influence of the Döderlein bacilli. Since it is generally possible to differentiate between free atypical nuclei, endocervical cell nuclei, and those of parabasal cells, an estimation of the existing hormonal status is easier than in bacterial cytolysis.

When smears show degenerative cell changes, it is advisable to note the presence or absence of Döderlein bacilli, as this gives a rough impression of the hormonal situation. When they are present one is almost certainly not dealing with a marked estrogen or androgen deficiency. Any other hormonal state is, however, possible. Smears, preponderantly showing free nuclei and partly recognizable cell structures in the absence of Döderlein bacilli, indicate an atrophic vaginal epithelium. Bacterial cytolysis of the more highly proliferated cells is rarely caused by organisms other than Döderlein bacilli.

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VIOLETTE M. NUOVO
Paris, France

CYTOLOGICAL FEATURES

The most salient feature of cytolysis is the presence of large colonies of Bacillus vag-

inalis. The epidermoid cells have fuzzy and ragged edges. Many free, naked nuclei are seen in every smear. Evidence of an inflammatory reaction may not be observed most of the time. In some cases it is observed only during the luteal phase of the cycle. However, most of the time it is seen during the entire cycle. In some of these cases, where signs of cytolysis were marked and chronic, smears obtained during the mid-cycle period, however, show minimal or no cytolysis. In most cases the Karyopyknotic and Eosinophilic Indices are very low. However, in a few cases they are normal.

In spite of the absence of cytological signs of infection, we resorted to local anti-infectious treatment in one of these cases on the assumption that proliferation of Bacillus vaginalis could be due to an infection. Treatment was pursued for 45 days, nevertheless, Bacillus vaginalis colonies and signs of cytolysis reappeared shortly after the treatment was interrupted.

CYTOLOGICAL STUDY OF CYCLES

A research program based on serial studies of vaginal smears performed during several menstrual cycles was started in our Department in 1950. We have since examined 3,157 patients. Cellular material aspirated from the posterior fornix was studied at least twice a week for a period of time varying according to the length of the cycle. Evidence of cytolysis was noted in 329 patients representing roughly 10% of our cases. This finding leads to a number of clinical and extra-clinical features which were observed in patients with cytolytic smears. We wish to submit these findings for further evaluation by other investigators. We will not discuss the problem of cytolytic smears seen in pregnancy as this is beyond the scope of this paper.

Interpretation of these types of cycles was difficult. Initially we considered them as compatible with a state of hypoestrogenism because of low Karyopyknotic and Eosinophilic Indices. However, it soon became obvious that this was an unsatisfactory explanation. At present, we believe this finding to be caused by a stepped-up output of androgens.

CLINICAL FINDINGS

Certain common traits in these patients soon came to our attention. We thereupon investigated a number of points which gradually acquired their proper significance, such as:

1. duration of menstrual cycles,
2. premenstrual syndrome,
3. mid-cycle syndrome,
4. temperature curves,
5. previous obstetrical history,
6. body hair distribution,
7. 17-ketosteroid excretion.

Unfortunately, we were considerably hindered in our efforts at collecting information and repeating laboratory procedures by the very peculiar psychic traits of these patients, who as a rule would not cooperate in regular work-up and follow-up schedule. Hence, information concerning average length of cycles was supplied in a fairly large number of cases, a much smaller number of patients agreed to draw up basal temperature charts and an even smaller number submitted urine specimens for 17-ketosteroid assays.

A fairly great number of patients had prolonged cycles or amenorrheic periods. This data was reliably ascertained in 268 cases. Ranges of from 23 to 33 days of cycle duration were considered as normal. Cycles exceeding 33 days in length were reported in 106 patients. Amenorrheic periods were noted in 64 cases. Normal cycles were reported in 105 cases and 11 patients had short cycles.

Patients reporting long cycles or amenorrheic periods represent 59% of the total case material. This is a significantly high percentage of patients with infrequent menstrual periods.

Another significant finding was the relatively high number of patients reporting premenstrual complaints. Among patients having supplied us with complete data and who presented evidence of total or partial cytolysis, 149 patients reported marked premenstrual symptoms, an overall incidence of 85%, compared to 26 cases with no complaints.

Mid-cycle syndromes were uncommonly reported; only 46 patients showed symptoms while 129 were symptomless - making for an overall incidence of 26% with this finding.

Most of the patients who agreed to draw up temperature charts at the time the smears were done, showed characteristic biphasic curves in spite of the absence of mid-cycle syndromes. A total of 62 patients (78%), had a characteristic temperature rise; only 17 patients had uninterpretable flat or oscillating curves.

Therefore, it seems probable that ovulation was taking place in all of these patients in spite of the lack of evidence of luteinization on the smears. A strikingly high number of patients had primary or secondary sterility. A previous obstetrical history was available on 121 patients with total or partial cytolysis. Of these, 40 were referred to our Department because of primary sterility and 8 because of secondary sterility. In all, 48 cases (39%), had been referred for sterility after repeated investigations had shown no other anomaly, such as tubal obstruction.

Of the latter 121 cases, 54 (44%) had had a spontaneous and unexplained miscarriage. Of these 54 cases, 22 patients had recurrent miscarriage resulting in sterility (in three cases with verified patent tubes); four other cases became sterile after an initial spontaneous abortion, and one patient had a stillborn child after an initial miscarriage.

On the basis of these past histories and having noted the slightly masculine psychic traits of these patients, our attention was also aroused by the high incidence of cases with an abnormal hair distribution. Pubic hair in most cases was of the feminine type but sparse tufts of hair were almost always found on the linea alba and surrounding the nipples. Downy upper lips and a particularly rich crop of hair over the legs completed this picture. We were able to do complete clinical studies in 95 cases; of these, 53 cases (55%) had a definitely abnormal hair distribution of the type described above.

17-Ketosteroid studies were performed in 38 patients. In 22 cases (57%), there was a definite elevation of the 24 hour excretion. In one case the excretion level was within the upper limit of normal and in 15 other cases the output was normal.

We are now studying the 17-ketosteroid excretion by the chromatic method of Mrs. Dingemanse.

Estrone, estradiol, and estriol studies were performed in four cases. They all had a normal level.

F.S.H. level was normal in the three cases studied.

Pregnandiol excretion level was normal in the eight cases studied.

These findings are summarized in Table I.

EXPERIMENTATION

I. Non-menopausal patients

Fifteen patients had been given androgens for a relatively prolonged period of time. These cases were eliminated from this series. Their cytological changes could have been induced by the administered exogenous androgens. One patient had had cycle studies done before which were considered to be consistent with a diagnosis of hyperestrogenism. A new series of smears made after testosterone treatment showed persistent cytolysis.

II. Menopausal women

A. Androgens:

Atrophic vaginitis: the administration of androgens to patients with atrophic-type vaginal smear had no effect on the vaginal smear.
Crowded menopause: in these cases the administration of androgens tended to have an atrophic influence on the vaginal smear.

B. Estrogens:

Atrophic vaginitis: with administration of low doses of estrogens the vaginal smear sometimes became cytolytic prior to the estrogenic type.
Crowded menopause: with administration of estrogen the vaginal smear became estrogenic in appearance.

TABLE I

	No. of patients with history *	No. of patients with treatment	Percentage
Long cycles (over 33 days)	286	106	37
Amenhorrhea	286	64	23
Premenstrual symptoms	175	149	85
Normal temperature chart	79	62	78
Primary and secondary sterility	121	48	39
One or several miscarriages	121	54	44
Abnormal distribution of hair	95	53	55
High level of 17-ketosteroids	38	22	57
Normal estriol, estrone, and estradiol level	4		
Normal pregnandiol level	8		
Normal F.S.H. level	3		

* Six of these patients were found to have a Stein-Leventhal disease

* None of these 329 patients had previous hormonal treatment

TREATMENT

We have attempted to treat these patients with a low dose of estrogens. In several cases we were able to do repeat smears which showed complete control of cytolysis. Most of these patients felt a very marked improvement of their premenstrual syndrome. Moreover, the period became more regular. Some of our patients became pregnant during this treatment and four patients who had had one or several abortions had full-term pregnancies.

In certain cases cortisone treatment was proved, with no results which could be in favor of the hypothesis of an ovarian origin of cytolysis.

COMMENTS

No definite conclusions may be drawn from this study, which only aims to direct attention to a hitherto unexplained cytological finding. In our opinion these patients show evidence of mild virilism (see Table I).

To illustrate this point we will compare one of our cases with a case published by Papanicolaou in 1939 in the March issue of "Endocrinology" under the title: "Suppressive Action of Testosterone propionate on Menstruation and its Effects on Vaginal Smears."

The photographs in Papanicolaou's paper demonstrate the sequence of events in the course of testosterone administration to an 18 year old female. They are:

1. disappearance of pyknosis and development of a hypoestrogenic aspect,
2. development of cytolysis with a dose of 125 mg of testosterone,
3. disappearance of superficial layer cells and suppression of cytolysis with ensuing androgenic aspect, presence of external basal and intermediate cells,
4. presence of internal basal cells, of atrophic cells, of erythrocytes and of polymorphonuclears, resulting in a typical aspect of atrophic vaginitis, with a dose of 750 mg of testosterone. After suppression of treatment identical aspects develop in inverse order.

The case chosen from our series was a 50 year old female. Two years after her climacteric, the patient, who had never received hormone treatment before, had a smear taken which showed signs of an atrophic vaginitis. The referring physician implanted 40 mg of estrogen and prescribed 100 mg of progesterone every month. Eight months later vaginal smears showed evidence of marked estrogen effect. Eosinophilic and Karyopyknotic Indices were approximately 50%. Androgen administration was therefore started, resulting in a correction of the eosinophilia and karyopyknosis. A cytolytic aspect was noted with an overall dosage of 350 mg of testosterone over a three week period. Androgen therapy was nevertheless continued and serial numbers of an androgenic picture with predominance of intermediate cells with a total dose of 920 mg of androgens.

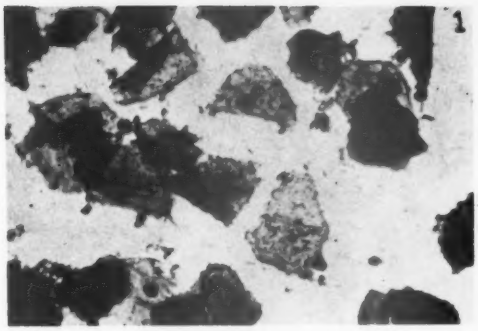

Fig. 1. Normal follicular phase before treatment, 9th day.

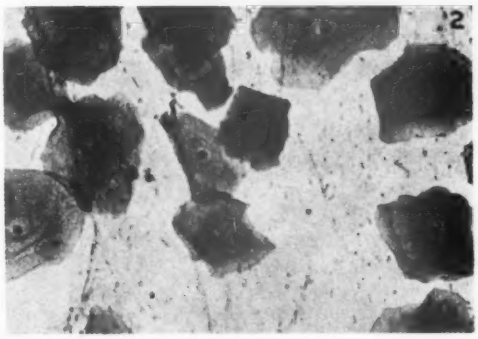

Fig. 2. Follicular phase induced with Progynon-B.

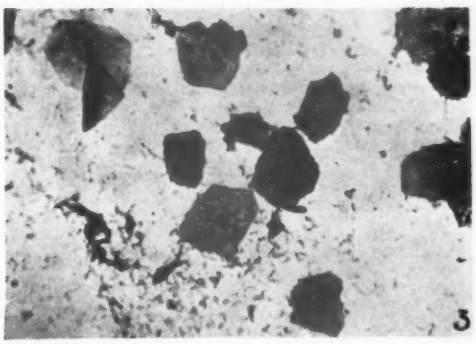

Fig. 3. Follicular phase, 10th day.

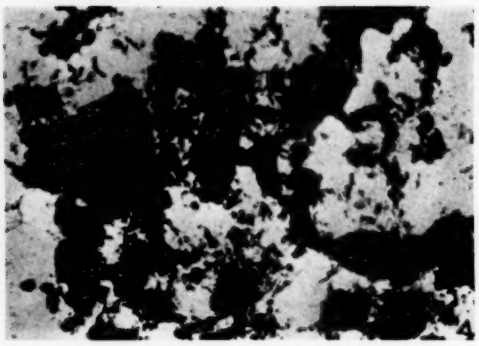

Fig. 4. Premenstrual phase with Bacillus vaginalis and pronounced cytolysis, 22nd day (11th day of treatment with oreton).

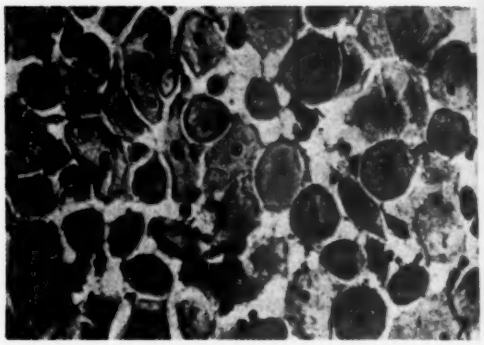

Fig. 7. Transitional (descending) smear type showing predominance of rounded compact cells and relative leukopenia ("Round cell type"), 11th day (22nd day of treatment with oreton).

Fig. 5. Menstrual phase, vaginal cells are of the large squamous type, 3rd day (14th day of treatment with oreton).

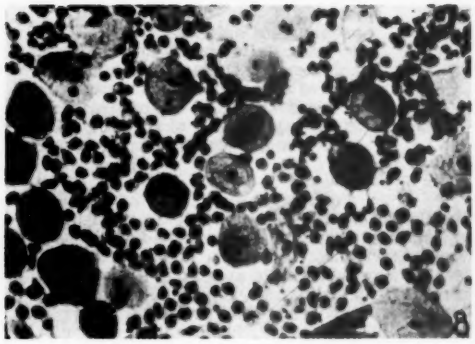

Fig. 8. Early atrophic or menopausal type with round cells and pronounced leukocytosis (Type 1), 12th day (23rd day of treatment with oreton).

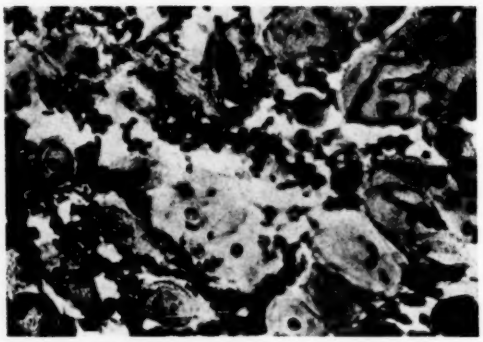

Fig. 6. Late menstrual phase, appearance of rounded deep cells, 7th day (18th day of treatment with oreton).

Personal observation: one sees that on Figure 4, after 10 days of treatment there is marked cytolysis.

ACKNOWLEDGMENT

The preceding photographs are from Papanicolaou, Ripley and Shorr, "Suppressive action of testosterone propionate on menstruation and its effect on vaginal smears," Journal of Endocrinology, 24: 3, March, 1939. Courtesy of Dr. G. N. Papanicolaou and Charles C. Thomas, Publisher.

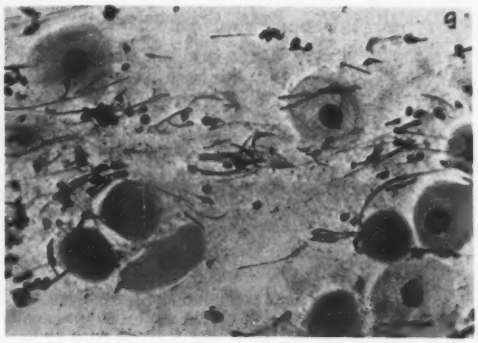

Fig. 9. Vaginal smear before treatment.

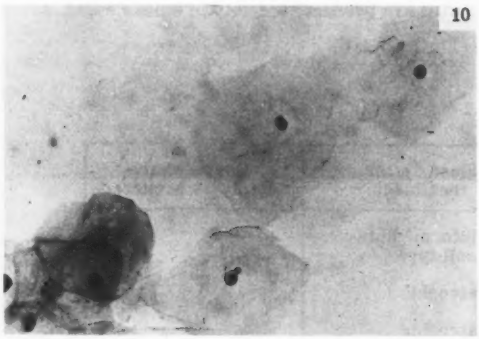

Fig. 10. Vaginal smear after estrogen therapy.

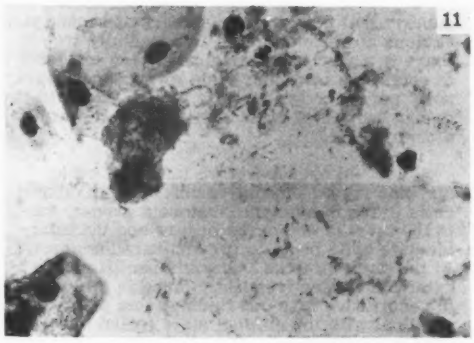

Fig. 11. Vaginal smear after androgen therapy.

Practical conclusions to be drawn from these cases are that the cytolytic aspects of the vaginal smear seem to reflect a degree of androgenic preponderance. However, the changes probably depend on the amount of excess androgen available, the amount of previously existing estrogen and the receptivity of the target organs.

The two cases reported would tend to prove that cytolysis occurs in one of the stages of the modification undergone by the vaginal cells during a stepped-up production of androgens.

It is an observed fact that patients with chronic cytolytic aspects show striking similarities in their clinical symptoms and general morphology. It is equally significant that they show habitual abortions and sterility, with only cytolysis to account for these anomalies. Cytolysis apparently reflects a latent and spontaneously developed state of hyperandrogenism which can be reduplicated experimentally by exogenous androgen administration, which also causes cytolysis.

DISCUSSION

PIERRE J. HAOUR, Lyon, Rhône, France:

Drs. Bickenbach and Soost give a very clear summary of cytolytic and autolytic conditions which can be differentiated mainly by the presence or absence of Döderlein bacilli, and by the type of cell affected.

I was already aware of the ideas of Violette Nuovo concerning the clinical aspect of patients with cytolysis, but I was very interested in reading her excellent paper and in finding there her condensed clinical data. We fully agree with her findings. We have also noticed the high percentage of prolonged cycles and amenorrhea. In our experience metrorrhagia was seldom found with cytolysis and we have never found cytolysis in cases with endometrial or cervical carcinoma.

We also obtained biphasic temperature curves although there was no appreciable quantitative or qualitative changes in the cytology.

I agree that cortisone does not change the cytolytic conditions (dosage used: 10 to 15 mg a day for 30 days). With estrogens we very often found some resistance of the cytolytic conditions (after 10 mg of estradiol dipropionate by injection or 2 mg of hexoestrol for 15 days). In several patients there was a non-significant increase of the Karyopyknotic Index with this dosage.

Concerning the hormonal stimulus in cytolysis, I am of the opinion that it is either a condition of estrogenic deficiency, which does not permit a normal differentiation of the superficial layers of the epithelium, or an inhibition of this differentiation through the intervention of a progestational or androgenic steroid hormone.

The cytology of the urinary sediment (urocytogram) is not affected by the same biological conditions as the vagina, and cells of the urinary bladder do not exhibit cytolysis. Therefore, their cellular integrity, in patients with cytolytic vaginal smears, permits the evaluation of the Karyopyknotic Index, taking into account

its lower value as compared with the vaginal Karyopyknotic Index.

Calculation of the Karyopyknotic Index in urinary sediment of patients with cytolysis has shown us a reduced mean value. On the other hand, a careful count in cytolytic vaginal smears, of Karyopyknotic Index cells which are always preserved gave us a still lower figure.

Therefore, the Karyopyknotic Index of the urinary sediment shows, as well as the vaginal smear, a lack of estrogenic stimulation on the receptive tissues.

HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D. F., Mexico:

We agree entirely with the paper of Bickenbach and Soost, and with the definitions of Wied, of "cytolysis" and "autolysis."

The observations of Violette Nuovo concerning the frequent findings of cytologists in cases of virilism or hyperandrogenism, are interesting. We have observed the vaginal smears of twenty women with mammary cancer that were receiving high doses of androgens. The results are summarized in Table I.

MOJMIR SONEK, Brno, Czechoslovakia:

In accordance with the statements of Bickenbach and Soost, we have found that the cytolytic smear cannot be regarded as a specific criterion either of the amount of estrogens or of any other hormonal effect. The very fact that this kind of smear occurs in diverse hormonal states (pregnancy, hypohormonal menstrual cycles, hypoestric amenorrhea, especially in various hormonal disturbances during puberty, etc.) testifies to a non-specific etiology of this state.

We accept the conditions for cytolysis as stated by most of the authors: presence of Doderlein bacilli in direct contact with an epithelial layer rich in glycogen, i.e., with the intermediate layer. We find this kind of smear in all states to which the above conditions apply. This is also the point of view from which we regard the statements of Violette Nuovo: The cytolytic smear cannot be considered a specific smear for androgen predominance, but a result of the suppressing action of the androgens on the estrogens. The proliferation of the vaginal epithelium can then remain on the level of the intermediate layer and, provided Doderlein bacilli are present, a cytolytic smear appears.

TABLE I

No. of cases	Smear before treatment	Smear after treatment	Cases where cytolysis occurred
1	estrogenic	intermediate cell type	1
1	estrogenic	atrophic	0
2	atrophic	atrophic	0
16	intermediate cell type	5 atrophic 10 same pattern 1 estrogenic	0 4 0
Total 20			5

All the patients were menopausal and none had received previous hormonal treatment.

From the results obtained, we can conclude with Violette Nuovo, that androgenic administration produces cytolysis. As Pundel, we believe that the cytolysis is not specific of any hormonal stimulus, and any hormone that produces the proliferation of the intermediate layer of the vaginal epithelium creates optimal conditions for the development of Döderlein bacilli and cytolysis.

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It is especially during prepuberty and puberty that the cytolytic smears are found under various conditions. This is especially true if there is a rise in the originally very low level of estrogens or if there is a drop in the originally normo or hyperestric level. The cytolytic smear here is of great significance in serial examinations.

In prepuberty we initially find an atrophic smear consisting chiefly of parabasal cells (often in autolysis) before a gradual rise in the level of estrogens occurs and the intermediate cells begin to predominate the smears. At the same time as Döderlein bacilli appear, cytolysis is also frequently found in this period. In accordance with our findings this picture is identical with the clinical state called fluor prepubertalis or pubertalis. In this case the cytolytic smear is a sign of a rising estrogen level.

On the other hand we can find the cytolytic smear in those cases in which the level of estrogens was originally high and is now dropping. This is especially true in hyperestric amenorrhea during puberty. This amenorrhea changes with bleeding or it can change over a longer period of time into a hypoestric amenorrhea. In the course of this change we find a decreasing number of superficial cells and an increasing number of intermediate cells usually undergoing cytolysis. The superficial cells resist cytolysis and therefore we find them as well formed cells in areas of cellular debris, free nuclei and Döderlein bacilli.

In conclusion, we can say that we believe that the cytolytic smear is not specific for any definite hormonal condition, but has diagnostic significance in serial cytological examinations.

CLOSING REMARKS

WERNER BICKENBACH and HANS-JÜRGEN SOOST:

The suggestion of Haour to examine the urine sediment in cases with marked bacterial cytolysis in the vaginal smears is good if the examiner has experience in urinary cytology. Urinary cytology shows some deviations from the vaginal cell patterns with regard to hormonal interpretation.

An advanced malignant process may be generally excluded in the presence of marked bacterial cytolysis (Döderlein flora) as has been often stated and was again mentioned in the discussions. The presence of bacterial cytolysis is not, however, an absolute proof for absence of malignancy. In our material of the last years, two cases of early invasive endocervical carcinoma and several carcinomas in situ were found with pure Döderlein flora and a more or less marked bacterial cytolysis.

THE VALUE OF EXFOLIATIVE CYTOLOGY IN THE DIAGNOSIS OF OVULATION

MARCEL GAUDEFROY
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It is commonly accepted that the basal body temperature is a good test of presumed ovulation and functional corpus luteum. We compare the routine vaginal smears of a menstrual cycle (one smear every day or every other day), with the basal temperature, and in difficult cases with the endometrial biopsy performed during the three days after the rise in temperature.

Without going into the details of our 10 years of experience, we consider, in general, the following conclusions as highly valuable:

1. The maximum of the Karyopyknotic and Eosinophilic Indices is observed just before the rise of the basal temperature, when the temperature rises quickly from one day to the next day. In these cases, the endometrial basal lucid zone appears two, three or four days later, at the same time that the vaginal smears exhibit regressive epithelial changes (clusters of folded intermediate cells).

2. When the basal temperature rises slowly over several days, the maximum indices are observed in the middle of the rising temperature and the endometrial subnuclear vacuoles are present from two to four days after the temperature has gone up. In these cases the vaginal epithelial regressive changes appear as soon as the temperature has risen, and a little before the subnuclear vacuolization of the glandular cells.

Among 44 particularly difficult cases, I have 8 cases of hyperestrogenism in which the peak of the Eosinophilic and Karyopyknotic Indices, exhibiting poor evidence of regressive epithelial changes, is reached only after a few days, followed by an increase of the basal temperature and the endometrial subnuclear lucid-zone. Perhaps these cases are of the type described by Ferin in castrated women who received a definite dose of a progestogen substance and a very high dose of ethinyl-estradiol (1).

Finally, I should like to report on the curious case of a young woman treated with 300 mg of testosterone propionate and immediately cured. She presented (seven days after the end of menstruation) an ovular cycle, with an increase in temperature and hyperthermic luteal phase, and also vaginal smears of the ovulatory type, without menstruation. Although the smears were of the estrogenic type and without an androgenic aspect, the common progesteronic criteria were clearly visible. (The endometrial biopsy was refused by the non-married patient). On the 16th day after the defective menstrual bleeding, a new normal cycle of basal temperatures and a new normal cycle of ovulatory type smears occurred, with presumed ovulation on the 16th day after the previously defective menstruation, and this time, the progesteronic phase was followed by normal menstrual bleeding (2).

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ABRAHAM E. RAKOFF
Philadelphia, Pennsylvania, U.S.A.

It should be emphasized at the outset that there is no known practical method which is absolutely accurate for determining whether ovulation has occurred. The various laboratory and clinical methods which are available are presumptive, and depend upon demonstrating changes in gonadotropins, estrogen or progesterone, or effects dependent upon these hormones. The "diagnosis" of ovulation from cytologic smears is therefore non-specific. It depends upon demonstrating the progressive rise in estrogen during the preovulatory phase to a variable peak level at the time of ovulation, followed by a more or less typical regressive pattern during the following two weeks, presumably caused by the combined effects of progesterone and estrogen and then their premenstrual fall.

No one single smear is "specific" for the ovulatory cycle, but rather only the sequence of typical phases is of significance in determining whether or not ovulation has probably occurred. Thus, increasing estrogen effect, by itself, is not specific for the ovulatory cycle, since it may occur in patients with follicular cystosis who fail to develop a corpus luteum. The "regressive" smear is likewise not specific for corpus luteum function, and may be initiated by a falling estrogen level alone or an increase in androgens. In my opinion, there is no single smear pattern which is specific for progesterone and the term "progesterone effect" is a misnomer. Even the proper sequence, of rising estrogen effect with subsequent regression, may be imitated in some anovulatory cycles by fluctuations in estrogen alone, or by estrogen and androgen. In such instances, however, there is usually a distinct difference in the sequential changes, particularly with regard to the following points: (1) in the ovulatory cycle the rise in the Eosinophilic or Karyopyknotic Indices is progressive but short-lived and does not plateau; (2) the regressive change following the peak is abrupt; (3) there is usually a persistence or even an increase in the eosinophilic cells which is maintained for a number of days before they almost completely disappear and further regressive and degenerative changes occur in the premenstrual phase.

The cytology smear is a more valuable technique for timing ovulation than for determining whether or not ovulation has occurred. In patients who, on the basis of previous observations, are expected to ovulate, the selection of the day of ovulation is based on the estrogen effect rising to a peak. The changes which precede this peak become evident several days beforehand, not only in the rising Eosinophilic and Karyopyknotic Indices of the vaginal smear, but also in the changes in the cervical mucus, evidenced by an increase in amount, liquefaction, "ferning" reaction, "spinnbarkeit" and decreasing cellularity. However, in my opinion, the peak effect is usually determined more sharply from the vaginal smear, on the criteria of peak Eosinophilic and Karyopyknotic Indices. In this respect, it is emphasized that smears taken from the lateral vaginal walls are more preferable for this purpose than those taken from the vaginal pool, which may represent cells shed a day or two earlier. The smear patterns of preceding cycles are valuable because individual patients show considerable variation in their peak effects, which although they may vary from cycle to cycle, are frequently in the same general range.

The cytology smear method presents certain advantages and disadvantages as compared to other methods, both in determining whether ovulation has occurred and also in "timing" ovulation, which may be seen summarized as follows:

The cytology smear, even if taken daily, is not as specific as the premenstrual endometrial biopsy in determining the presence of progesterone effect, and therefore, indirectly, the occurrence of ovulation. I would also place more reliance on a typical biphasic temperature chart showing the characteristic 12 to 14 day progesterone-induced temperature elevation than on the cytology smears. On the other hand, if the chart is slow-rising, or erratic, or if the patient is unreliable in this technique, then I would place precedence on the information provided by the smear. Pregnandiol excretion levels in the urine are of limited value for routine use, in that they are too time-consuming and expensive to be done in daily routine fashion and single determinations are subject to considerable fluctuation.

The vaginal smear method, in my opinion, is usually the most satisfactory technique for timing ovulation because of its sharp end-point and its simplicity.

It is apparent that the smear method used in conjunction with other techniques affords the clinician the most satisfactory results. A combination of daily or near-daily smears, cervical mucus tests, basal temperature charts and endometrial biopsy provides the best results.

HERBERT RAUSCHER

Vienna, Austria

In the biphasic cycles one can recognize, with regularity, a period of time which may be identified as the pre-ovulatory phase (1). This pre-ovulatory phase is reflected in the vaginal smear with a definite smear pattern. The cellular changes which occur during this period of time may be interpreted as an expression of the increase of estrogenic stimulation. The beginning of the pre-ovulatory phase may be identified if, under daily smear control, one finds a pattern which shows the following criteria: 1) tendency towards isolated positioning of the superficial cells, and 2) increased amount of apparently freshly exfoliated, flat, eosinophilic superficial cells with homogeneous, dark, centrally located, small to point-like nuclei.

The cytological pattern which is characteristic for the beginning of the pre-ovulatory phase remains present until the last day of this phase. One finds only a slight change in individual cells, from which one can conclude that the estrogenic stimulation persists which is responsible for the pre-ovulatory changes. This persistency of estrogenic stimulation can be recognized from an increase of the size and the transparency of the eosinophilic cells and also from an increasing softness of their contours. The increase of the percentage of the eosinophilic cells may be used only as an indication for the persistence of the estrogenic stimulation if the individual morphological features of the cells correspond with this increase of estrogenic activity. The cytological diagnosis must rely on the general pattern of the smear as well as on individual cytological criteria. The cytological evaluation is considerably easier if one has the opportunity to compare the smear finding of two consecutive days.

The cytological determination of the end of the pre-ovulatory phase can be made on the basis of cytological signs, or patterns, which express the decrease of the estrogenic stimulation. We can assume that the last day of the pre-ovulatory phase is the day which precedes immediately the first day on which we find these cytological signs of decrease of estrogenic stimulation. The decrease of the high estrogenic activity which had been characteristic of the pre-ovulatory phase effects, first, the turgor of the cells. The decrease of the turgor

of the cells can be recognized, at first, by shrinkage of the eosinophilic cells. The decrease of the turgor is documented by the occurrence of a more definite cell membrane which had previously been very fine and hardly recognizable. It is also to be recognized, by means of the decrease of the cellular transparency and, finally, by a more dull staining reaction which replaces the previously rather bright staining of the cells. Subsequently, the regressive phenomena on the cells, which may still lie isolated, becomes more and more marked, so that 24 to 48 hours after the appearance of the loss of turgor, pictures arise which allow one to easily recognize a decrease in the estrogenic stimulation by a folding and crowding of the eosinophilic cells. All important details pertaining to diagnosis of the preovulatory phase have been described elsewhere. We consider treatment with appropriate antibiotics prior to taking the smears, as a necessary prerequisite for a clinically valuable diagnosis. We use 50 mg Terramycin tablets, inserted intravaginally once, two days before the series of smears is to begin. After 48 hours, cellular debris, bacteria and the majority of the leukocytes have usually disappeared so that from that time on pictures appear which disclose clearly the criteria necessary for diagnosis. After this pre-treatment the beginning of the pre-ovulatory phase can almost always be determined on a certain day. The end of the pre-ovulatory phase, as announced by decreasing estrogenic stimulation, can be determined that exactly only in a fraction of the cases. In the remaining cases the decrease of estrogenic stimulation can be fixed only within a time period of 2 days.

In the vast majority of the 1023 menstrual cycles which we examined by daily vaginal smears we could find a pre-ovulatory smear type on either two or three days, whereas, in the remaining minority of the cases the pre-ovulatory smear type was found on either one day or on four days.

During laparotomies which were performed at the time when the vaginal smears showed the pre-ovulatory pattern we could obtain a series of corpora lutea (3, 4) each of which could be identified by means of histology to be not more than 24 hours old. According to these findings it was natural that the corresponding endometria showed only proliferation. These operations have been performed within a period of 2 to 12 hours after the first signs of decrease of estrogenic stimulation appearing subsequent to the pre-ovulatory phase.

For research purposes it is necessary that we can identify the beginning of the pre-ovulatory phase as well as the end of the phase and fix these periods on a definite day. However, for practical purposes it is sufficient to identify the onset of the phase. The pre-ovulatory phase never lasts longer than four days (5). One knows, therefore, on the day when the pre-ovulatory smear pattern occurs that (a) the period in which ovulation may occur is imminent and (b) the time period must be terminated in four days. The examination of various cycles of the same patient is made easier due to the fact that they type and the duration of the pre-ovulatory changes are relatively constant in one individual patient.

The only real criterion for the occurrence of ovulation is the occurrence of pregnancy. Ovulation cannot be diagnosed definitely by means of the cytological smear, nor can it be diagnosed by any other indirect technique. It is my opinion that the resulting progestational effect cannot be definitely identified by means of the smear because progesterone is an individual substance. Neither will it produce any specific effects in simultaneous action with estrogen and, therefore, the indirect conclusion that ovulation has occurred can, by means of the smears, only be suggested. Such conclusions seem highly justified when, before the appearance of these cytological patterns, characteristic for the second half of the ovulatory cycle, a typical sequence in the pre-ovulatory phase can be observed. From this we conclude: the cytological examination has only a problematic value in identifying whether or not ovulation has occurred. The cytological examination, is , however, rather reliable in the identification of the time period in which ovulation can occur. This time period begins, at the earliest, on the last day of the pre-ovulatory phase and is, at the latest, terminated with the end of the following day.

We consider the cytological examination for the determination of the ovulation time as a necessary part of the examination. However, in practice the cytological exam should not be relied upon as the only technique but should be combined with a macroscopic control of the cervix. We have called this type of approach "simultaneous examination of smears and cervix" (2) and we could show that with the application of both techniques one could identify the ovulation time and the optimum time for conception (4, 6) with the highest degree of accuracy.

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ERICA WACHTEL

London, England, U.K.

Vaginal smears taken from a normally ovulating woman at different stages of the cycle differ from each other sufficiently to allow conclusions on the state of the ovaries and the endometrium. The follicular phase of the cycle is characterized by a gradual straightening up of the epithelium with an increasing percentage of eosinophilic superficial squamous cells, a steady rise in Karyopyknotic Index readings and a gradual decrease in leukocytic and bacterial content. At ovulation, the smear is characteristically free of leukocytes and almost free of bacteria, but may show a few red blood cells;

the epithelial cells are arranged singly and their edges are flat and clearly outlined. The Karyopyknotic Index has reached its peak value. Although this pattern is always present at normal ovulation it is not diagnostic of follicle rupture since it merely represents the response of the vaginal epithelium to estrogen. This pattern is, therefore, to be found in women with persisting follicles, in the presence of estrogen producing tumors, following the administration of estrogens in postmenopausal women as well as in surgical castrates, etc. For this reason, it would appear premature to diagnose ovulation from the presence of an ovulatory smear pattern. The cytologist may only be certain of normal ovarian function when the ovulatory gives way to the secretory pattern in the absence of progesterone medication. The latter is characterized by clustering of cells, folding of cell edges and a pronounced influx of leukocytes and micro-organisms.

Whereas proliferative and ovulatory patterns may be simulated by estrogen administration or excess endogenous estrogen, inflammatory conditions as well as technical factors such as inadequate spreading of the vaginal secretions may be responsible for errors in diagnosing secretory patterns. Recognizing these pitfalls it seems a wise precaution in order to avoid falling into them to study the smear patterns of a whole cycle at repeated intervals.

DISCUSSION

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

We approve of the critical way in which all of the main speakers explained this subject. We interpret the cytological cell pictures exactly as Rauscher does. The cytological examination is certainly still the most satisfactory method for determination of ovulation, none of which are 100% reliable. Nevertheless, three points have to be considered: 1) ovulation can be foreseen only within a period of one to two and probably three days; 2) one can only suspect within the first 24 hours after ovulation, according to the early signs of progesterone effect, when ovulation really occurred; 3) it is not a specific test for ovulation.

Undoubtedly it is best to use, in addition, more methods at the same time, e.g., macroscopic observation of the external os, increase in mass of cervical mucous, its transparency and its quality for spinning, the formations of fern crystals and the basal temperature. We especially do not want to miss the basal temperature. It appears to us not safe enough in determining ovulation, but safe for determining the question of whether or not ovulation has occurred, than the examination of smears.

Especially we want to emphasize the requirement of Rakoff for taking the smears from the lateral fornix of the vagina. It is important for such subtle examinations, such as the determination of ovulation, to obtain technically absolutely incontestable smears. Fresh cells from the surface of the epithelium should be examined which are most successfully obtained from the lateral fornix of the vagina. In the posterior fornix there are usually to be found cells which have been exfoliated long before and have accumulated there. Smears from different spots of the vagina may differ essentially from one another as far as degree of proliferation and folding and accumulation of cells is concerned. The signs of progesterone effect are often less distinctively recognized in smears from the ectocervix than in ones from the lateral wall of the vagina. Therefore, grave errors may result if the smears are taken from different spots on different days.

It is, of course, important that series of smears are taken daily beginning on the eighth to tenth day of the cycle and continuing on ovulation. In many cases judgment will only be possible if the Doderlein flora temporarily is eliminated by insertion of an antibiotic into the vagina.

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JEAN A. de BRUX, Paris, France:

I fully agree with the main speakers' assertions. The only true criteria of ovulation is pregnancy, or the finding and the picking up of the ovum itself on the ovary during coelioscopy, as Palmer has often succeeded in doing.

All other indications considered as tests of ovulation are in reality merely presumptions, very strong in certain cases, but presumptions nevertheless.

This being granted, the smear is one of the best methods for determining the presumed date of ovulation, provided that daily smears are examined during the peri-ovulatory period, in a non-inflamed vagina.

I am surprised that none of the main speakers made allusion to the "rolling in" of the cellular edges. In my opinion, this is the first sign, not of ovulation, but of the diminution of estrogenic action, corresponding to the 24th day after ovulation.

But the vaginal smear cannot show everything, for the vaginal mucosa is too sensitive and too inconstant; its states are momentary rather than cumulative as in the case of the endometrium. Hence, for an exploration and treatment of female sterility connected with disturbances of the cycle, it is necessary to associate the cytologic and the histologic methods, the latter being the only valid means of appreciating the nidation potentialities of an endometrium.

PIERRE J. HAOUR, Lyon, Rhône, France:

I fully agree with the authors that in order to obtain a diagnosis of ovulation by means of cytology, it is necessary to perform several smears in order to study the changes in the qualitative and quantitative patterns of the cytology, which represent the only criterion of ovulation. I agree also that the increase of Karyopyknotic and Eosinophilic Indices as seen in midcycle is not diagnostic for ovulation, but if regressive changes follow, they are specific for progestational effect. Groups of folded cyanophilic cells are also encountered in the first weeks of the cycle. However, as we could find by temperature charts and by endometrial biopsy, when the cellular groups are properly spread throughout the smears and contain cells with a pale, loose and stringy cytoplasm, this pattern most of the time was correlated with the production of progesterone.

In our experience also, smears of the urinary sediment in the diagnosis of ovulation are easier to interpret: there are fewer elements in the smears, and inflammatory conditions are not as frequently observed as in vaginal smears. Thus, changes related to ovulation are more evident: in the luteal phase of the cycle, groups of cyanophilic cells are not folded but sometimes appear as sheets of pale juxtaposed cells which seem to be under the control of progestational stimulation.

Urinary smears are also useful in cases of cytolysis: cyclic changes are observed in the urine, though most of the time it is not evident in cytologic vaginal smears because of existing cytolytic changes.

The difficulty of the diagnosis of ovulation by cytology remains blocked because of the necessity of obtaining a series of smears and I would like to ask the authors, especially Rauscher who examined 1023 menstrual cycles by daily smears, which method they employ for collecting their smears. Do they let their patients perform the smears themselves?

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

Serial vaginal smears taken from the uninfected vagina of the normally ovulating woman show a specific cycling consisting of peak Eosinophilic and Karyopyknotic Indices with absence of leukocytes at the time of ovulation, succeeded by "regressive" changes comprising folding, clumping and mucification of cells from slightly deeper layers. As Rakoff emphasized, however, the vaginal smear changes are only presumptive and indirect.

The cytologic technique is not for the busy clinician who looks at smears only occasionally; it presupposes training and interest in cytology. Utilized with other methods, however, it adds confirmatory evidence that ovulation has occurred.

ALEXANDER MEISELS, Mexico, D.F., Mexico:

When discussing the value of exfoliative cytology in the diagnosis of ovulation, special emphasis should be given to the technique used. In more than 700 menstrual cycles studied, I have found that even slight deviations from the usual technique account for important mistakes. For several years I have been following Pundel's technique and have obtained very good results with it. Smears are always taken with a wooden spatula from the upper third of the right lateral vaginal wall and are stained with the Shorr-hematoxylin method. Complete differential counts are done and progesterone activity is evaluated taking into account the great clustering of cells, with unclear membranes inside of the cluster, and the pronounced folding of the cells.

Cycles are studied with smears taken every day or every other day. In order to compare the results of the cytologic studies, and to complement them, cervical mucus is studied for its Spinnbarkeit and "ferning." Many of our cases were also followed with basal temperature charts and in some patients, endometrial biopsies were studied in the premenstrual period.

When the cytology shows the classical cyclical changes and the mucus studies correlate well, we feel that we have enough data to diagnose that an ovulation has occurred. Nevertheless, in order to give an approximate date on which ovulation occurs, we feel that it is necessary to study a second cycle, and we give the information only if this second cycle shows a pattern comparable to the first one, in which ovulation was demonstrated. This is important, mainly for sterility cases, when artificial insemination is planned.

In two cases, the ovulatory cycle studied was followed by pregnancy. This gave us an opportunity to follow the very early pregnancy by daily smears, and the cytologic observation was continued into the fourth month in both cases. In both patients, the second half of the cycle showed a marked progesterone activity which kept increasing as the Karyopyknotic Index fell. In the fourth week a few "navicular" cells appeared and their number increased slowly during the next weeks. Very soon, the smears showed a pregnancy-type picture and in both cases, pregnancy was suggested from cytologic data before biologic tests became positive.

If cytologic technique is meticulous and correlated with studies of the cervical mucus as well as with clinical data, a diagnosis of ovulation can be expected to be correct in the overwhelming majority of cases.

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GIUSEPPE MOGGIAN, Bologna, Italy:*

The systematic adoption of vaginal cytology by the Obstetric and Gynecology Clinic of the University of Bologna in all endocrine dysfunctions has allowed several aspects of the diagnosis of ovulation to be considered.

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The Sterility Center, connected with the clinic, annually receives many married couples that undergo hysterography and uterine tube insufflation, in addition to the following methods:

a) vaginal smears on alternate days for two cycles,
b) charting basal temperature for three months,
c) cervical mucus tests,
d) endometrial micro-biopsy

The difficulty of not being able to perform all of these tests in every case notably modified the diagnosis and therapy.

These examinations, along with many years of experience in cytology, enables us to draw the following conclusions regarding the diagnosis of ovulation:

1. Cytological examination permits, in 60-70% of the cases, the determination of the date of ovulation within 24 hours. It is in these typical cases that the pre-ovulatory phase is followed by the classical cytological picture consisting of cellular elements, mostly eosinophilic which are flat, isolated and contain pyknotic nuclei. The confirmation of the beginning of ovulation is obtained within the following 48 hours, since the regressive modifications appear which are characterized by the drop in estrogens (Pundel, 1950) or as are seen in the progesterone phase. In the remaining 30-40% of cases due either to the transient quality of the ovulatory picture or to the presence of other interrelated hormones (androgens, adrenal hormones, persistent corpus luteum, etc.), the cytological changes are not characteristic and it becomes impossible to determine the date of ovulation merely by the cytological examination.

2. When the endometrial biopsy was employed it permitted the establishment of post-ovulation but not the date of ovulation as precisely as the cytological method. We have employed the endometrial biopsy as a control when using the cytological examination and although the microscopic picture was always similar it was not confirmatory either by the intensity of the proliferation phenomenon or by the stromal behavior.

The cytological and histological picture confirmed each other in 98% of the cases. In the remaining 2% there was no confirmation, in as much as the endometrium appeared to be still in the postmenstrual phase.

3. Basal temperature constitutes a test of primary importance in determining the period of ovulation, although it must be considered with some reservation (1). The use of this method in most of the cases which we observed allowed us to establish, in accordance with Palmer (2), that ovulation in the majority of cases coincides with the end of hypothermia. In fact, basal temperature being the expression of the level of the progestative hormone we can say that only in the presence of a sufficient quantity of the luteinizing hormone will it rise. In a certain percentage of cases involved in our study (from 1-43%) it was not meaningful; either because the biphasic character of the curve was missing or due to a gradual and progressive climb.

4. Regarding the crystallization of cervical mucus in the ovulatory phase, described by Raucher (3), Rakoff (4) and Pundel (1), we are able to observe that these modifications are quite variable in time, from subject to subject and cycle to cycle, as these are the expression of the estrogenic level of the body. In fact, in about 62% of the cases, the maximum secretion of the cervix corresponds to the Eosinophilic and Karyopyknotic Indices while in the remaining 38% there was no confirmation within 72 hours.

From what we have been able to observe, we are in agreement with Rakoff (4) that one individual cytological examination for the diagnosis of ovulation is insufficient. As a matter of fact, we have obtained specimens on alternate days, making certain that the variations of the cytological pictures found in the second smear, represented a typical sequential change which suggested the occurrence of ovulation, which would not have been possible from the first specimen alone.

Under this procedure we disagree with Rakoff (4) as to the possible cause of error in ovaritis, for even though there exists a certain variation in the cytological picture, it differs from the ovulatory picture. The latter is determined by hormones, in addition to other factors of a neuro-vegetative nature, that act on the genital apparatus. Regarding the confirmation of the beginning of ovulation in that period, even taking into consideration the existence of an abrupt fall of the indices in the post-ovulatory period, the diagnosis of the beginning of ovulations always remains problematical, according to Rauscher (3). Even with endometrial biopsy carried out in the premenstrual phase, the persistence of the corpus luteum could falsify the histological diagnosis. The association of cytological research with basal temperature seems very significant. In fact, there is a correspondence between the beginning of regressive phenomena and the rise in temperature, when the latter presents the characteristic biphasic progress, according to Gaudefroy (5).

In addition, daily samplings in these cases eliminates the causes of error (6).

Studies of cervical mucus were also carried out in conjunction with vaginal smears. However, the incidence of error appeared to be too high to be of any aid in the diagnosis of ovulation. Vaginal phlogistic processes, hyperestrinism, pelvic congestion of various causes, could considerably alter the aspect and quantity of a complete response, as was obtained with other methods.

In conclusion, the vaginal smear has been found to be the most precise and easiest method, as regards both obtaining the specimen and cost, as a routine method. In conjunction with basal temperature it presents a good technique. Logically used in conjunction with other methods, it allows a more exact study of the conception period of the female.

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HÉCTOR MUNGÚIA and ESTHER FRANCO, Mexico, D.F., Mexico:

Our experience acquired in nine years of cytologic work confirms exactly the papers of the main speakers.

We would only like to mention that the ovulation as such is not reflected by any specific cytologic change. It is the ovarian follicle and the corpus luteum that give the indirect cytological signals of its probable occurrence, through the action of the follicular and luteinic hormones.

All other procedures are also based on indirect evidence that ovulation has occurred. In a woman in whom the occurrence of ovulation has been established by previous observations, vaginal cytology has a great value in the determination of the time of ovulation.

J. PAUL PUNDEL, Luxembourg, Luxembourg:

I am very glad to see that the conclusions of the main speakers are so concordant and that my own experience based upon the complete study of over 1000 patients seen for sterility or functional menstrual disorders permits me to agree completely with Gaudefroy, Rakoff, Rauscher and Erica Wachtel. As I have presented my own findings elsewhere (2, 3, 4), I would like to give, at this time, only a few conclusions.

1. The so-called pre-ovulatory smears or the ovulatory peak of the Eosinophilic and Karyopyknotic Indices are in no manner specific for ovulation, since the same smear pictures can be observed in anovulatory cycles. As in nearly all other tests for ovulation, such as basal temperature, endometrial biopsy and pregnandiol determination, the vaginal smears permit only a presumptive diagnosis of ovulation, because all of these tests are based upon the different effects of progesterone. They permit only retrospective conclusions if progesterone effects have been identified. This is possible only if ovulation has occurred with formation of a functional corpus luteum, but not before.

2. As I have shown in other papers, the progesterone effect in the vaginal smears can be identified only if there appear true clusters of folded intermediate or superficial cells. Therefore, the first regressive changes of the vaginal smears (the pictures of the immediate postovulatory phase from the 15th to the 17th day of a normal cycle) which show mostly the appearance of folded cells, but no clusters, are in no manner specific for a progesterone effect or of ovulation, since these regressive changes can be observed also in anovulatory cycles. The presumptive diagnosis of ovulation by vaginal smears can be posed, therefore, only after the 17th day, or two to three days after the ovulation has occurred, when the progesterone effect on the vaginal cells is sufficiently strong to be identified as such.

3. Some authors, especially Moricard (1), do not accept the possibility of diagnosing progesterone effect by vaginal smears. The vaginal smear is for these authors, a priori, rejected as a helpful test for the diagnosis of ovulation or of the progesterone effect. With Vokaer (5), I think that this conclusion is too exclusive for the following reason: If in a sufficient number of menstrual cycles we find a concordance of nearly 90% between the findings of vaginal smears, basal temperatures, endometrial biopsies and pregnandiol determinations we have to accept it as a biological fact. If in experimental cases one could possibly reproduce similar vaginal changes by other hormones than progesterone, I think that this conclusion of the non-specificity of the so-called progesteronic vaginal reactions will not reduce the practical value of the vaginal smears in the study of the menstrual cycle or its spontaneous disorders.

From these facts, I would conclude for practical purposes the following: If one has to study the real progesterone effect in the main organ for which progesterone is physiologically secreted, one should study this organ directly, (the uterus), and the best test is the endometrial biopsy. But the latter, as it is generally taken in the immediate premenstrual phase, permits no conclusion concerning the day of ovulation. If we would like now to find out the day of ovulation, we would have to rely upon the basal temperature and the vaginal smears. But both tests give only a retrospective diagnosis and there remains, in both tests, a period of two to three days where they permit, in many cases, no positive conclusions. However, the vaginal smear, in my opinion, permits the most accurate diagnosis, since the postovulatory changes in the smear are preceeded by a rather typical smear type of short duration. If in a cycle the so-called ovulatory peak of the smear is followed by the typical regressive changes of the postovulatory phase, one can conclude, with rather complete certainty, that ovulation did occur on the day of the ovulatory peak. Exceptions are very rare and mostly due to inflammatory reactions of the vaginal content or to inadequate collection technique of the smear.

The vaginal smear is, therefore, in my opinion, the most satisfactory test for timing ovulation; but for a complete endocrinological evaluation of the menstrual cycle, especially in cases of sterility, it must be associated with the other usual tests such as endometrial biopsy, basal temperature, etc. However, the vaginal smear offers valuable results only if the smears are repeated, preferably every second day and are taken correctly, not from the posterior vaginal pool, but from a lateral wall of the vagina.

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MOJMIR SONEK, Brno, Czechoslovakia:

Our findings agree with those of Rakoff. The rise of Karyopyknotic and Eosinophilic Indices is not in itself a specific sign of ovulation. Very often we found the rise of the estrogenic indices to be identical with the normal follicular phase and a typical "preovulatory picture." This peak was not followed by any secretory changes, but by a hyperestric plateau. This plateau ended a few days before menstruation with a sudden decrease of the estrogen level accompanied by pre-menstrual degeneration of cells. That is the most frequent type of anovulatory asecretory cycle.

The question of determining the specific progesterone effect in the second half of the cycle is very difficult. The curling and folding of the cells described by most authors in the secretory phase can be also the consequence of the decrease in the estrogen level. We believe that an experienced cytologist can differentiate the changes found in the secretory phase from those resulting from the hormonal decrease. However, without the possibility of strict specification of this difference, the scientific value of cytological diagnosis of the secretory phase is problematic. For practical purposes we have laid down the following criteria for the diagnosis of ovulation:

1. The well-developed preovulatory phase.

2. The post-ovulatory changes are sudden and they are more concerned with the morphology of the cells (typical folding and curling) than the decrease of estrogenic indices. This decrease is prolonged and not very marked.

Only the normoestric, well-developed biphasic cycle and the typical hyperestric anovulatory cycle can be diagnosed with relative accuracy. The cytological diagnosis of the other conditions, especially the hypohormonal biphasic cycle and anovulatory cycle with an atypical course of estrogenic indices is very difficult or even impossible, and must be accompanied by other methods (basal temperature, crystallization test, biopsy).

The cytological diagnosis of ovulation must be complex, it can be stated only in typical cases and it is usually retrospective.

GUILLERMO TERZANO and ARTURO A. ARRIGHI, Buenos Aires, Argentina:

It is known that all the methods now available for detecting ovulation in the human female are not perfect. We agree with the statement that serial vaginal smears "is a more valuable technique for timing ovulation than to determine whether ovulation has occurred," because a great deal depends on subjective appreciation of minute variations. Generally it is not difficult to evaluate estrogenic effect by vaginal smears during a normal follicular phase, nor to evaluate a normal progestational phase, but it is not easy to make correct diagnoses in the presence of borderline cases when the picture of progestational effect is insufficient or poor. "Factors responsible for errors" have been discussed elsewhere in ACTA CYTOLOGICA.

Out of 72 cycles reported as ovulatory in our laboratory, 59 biopsies showed progestational endometrium and 13 did not. It gives an accuracy in our hands of only 83.3%.

We should not ask of a good method more than it can give, and we still rely on endometrial biopsy. Nevertheless, when an undesired sterility makes anovulatory cycles pathologically significant, serial vaginal smears used together with basal body temperature, "spinbarkeit" and crystallization of the cervical mucus (all checked with endometrial biopsy) may become an excellent tool in the study of the menstrual cycle and will increase the dependability of the smear test in determining and timing ovulation in women.

CLOSING REMARKS

HERBERT RAUSCHER:

The Symposium has brought out general agreement, especially concerning two facts:

1. With certain technical presumptions, the daily examination of vaginal smears represents actually the most satisfactory test for detection of the time during the current menstrual cycle in which ovulation may occur. For practical purposes we would like to add that detection of ovulation-time often is made much easier by association of the simultaneous macroscopical observation of the cervix. Our respective procedure is restricted to the determination of the daily changes in three factors: (a) size of the os, (b) the quantity and (c) the transparency of the mucus. Examination of "Spinnbarkeit" is only rarely done by us; examination of the crystallization was discontinued years ago.

2. The question, whether or not ovulation did occur, may be answered far more reliably with account from basal temperature charts, endometrial biopsies and pregnandiol determinations.

From the foregoing one may conclude: When time of ovulation is of primary importance, as for certain diagnostic and therapeutic procedures in sterility, examination of cytologic smears and also of the cervix are indispensible. A great advantage of the simultaneous examination, which is a test based on the effects of estrogen activity, consists in the fact, that ovulation time during the current cycle is announced in advance. When, however, a functional disturbance is to be ascertained, that is, whether or not a woman's ovulatory cycle exists or not, tests based upon specific progestational effects must be regarded as more useful than smear examinations. We feel, that

this should be considered especially true from the scientific viewpoint. For clinical application the so-called "postovulatory" reaction in the smear picture will often be sufficient, especially if combined with a well recognizable regression in the functional behavior of the cervix.

In order to prevent any possible mistake, we would like to say that by the term "preovulatory phase" the rupture of follicle in no manner should be prejudicated. Considering the results of our comparative investigations concerning the relations between the ovary and the behavior of receptors demonstrating estrogen activity, we may only establish the following: If ovulation does occur during a cycle, then it takes place at the end of a short lasting phase, which is characterized by the signs of a transient increase in estrogen activity; we feel, that the typical smear type as seen during this phase therefore may be called "preovulatory."

We completely agree with those authors, especially with Bickenbach and Soost, who emphasize the importance of technically incontestible cytologic smears. As already said, pretreatment with an appropriate antibiotic is for us indispensible for the examination of smear series during the cycle. We too take the smear from the lateral vaginal wall and employ for that a platinum loop, since we believe that by doing so the resulting smears are, as far as possible, free of undesirable contamination. Smears taken by the patients themselves, which we examined for a longer period years ago, were rather disappointing from a diagnostic point of view.

ERICA WACHTEL:

I would like to assure Sonek that serial vaginal smears are of value not only in establishing whether or not ovulation has occurred, but also in the investigation of anovulatory cycles. Persistent follicles are only one reason for failure of ovulation. Vaginal smear patterns in varying degrees of ovarian failure are interesting and most informative as demonstrated by Ines de Allende and Pundel, since they give an indication as to the degree of the ovarian disturbance and the choice of treatment.

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THE VALUE OF EXFOLIATIVE CYTOLOGY IN THE DIAGNOSIS OF FOLLICULAR PERSISTENCY

JEAN A. de BRUX
Paris, France

At both extremes of the female genital life, and sometimes even in normally menstruating women, there may occur disturbances of the cycle whose cause is not always clear. Such disturbances may sometimes be traced to the persistence of one or more graafian follicles.

The diagnosis of this persistence is difficult and should be supported by endometrial biopsies concurrently with a careful study of smears during one or several cycles.

One of two characteristic cytological aspects - at the outset apparently very different - may be found:

A. a sustained, high follicular stimulation, or

B. a persistent, moderate follicular stimulation by "follicular inertia."

A. High and sustained follicular stimulation

This state is found in puberty and in the pre-menopause and is accompanied by menstrual irregularities. The smear series shows an ovulatory aspect, with high levels of pyknosis and eosinophilia. A few days before the onset of the menses (which may be either very abundant or, on the contrary, very scanty), there is noted a slight reduction of the pyknosis and the eosinophilia, with tendency to agglutination. Then, with the menses terminated, these levels again become and remain high.

This hyperfolliculinism may be interpreted histophysiologically as resulting from the successive growth of several follicles or by the persistence of a single follicle (sometimes both simultaneously).

At puberty, the follicles grow rapidly and then regress. One follicle, however, reaches maturity, even though the ovum has already degenerated at the start, but it does not erupt, and conserves its functional activity for a rather long time, then slowly regresses. In the meantime, a second follicle reaches maturity, adding its activity to that of its predecessor (Fig. 1).

Clinically, this may be manifested either by an amenorrhea, if the estrogenic level remains high, or by hemorrhage - sometimes extremely severe - due to hormonal privation.

The endometrium shows a hyperplastic aspect, with numerous mitoses on the epithelium of the glands, as well as on the stroma. In the menopause, the aspect may be identical, but often enough such hyperplasia is (focal), the formation being "anchored" to the deeper part of the endometrium by thick-walled and sclerotic vessels.

B. Persistent, moderate follicular stimulation by inertia of the follicle

The series of smears shows a slow progressive rise in the level of the superficial cells, but the level of superficial cells with pyknotic and eosinophilic nuclei never exceeds 20 - 30%. Later, after a period of varying length, and without any definite elevation of ovulatory type, the cells undergo an increasingly marked cytolysis, with cellular debris and agglutinated masses. The aspect is that of a luteal (or rather, pre-menstrual) smear, but there is no menstruation. On the contrary, the smears become clean, the cytolysis disappears, and there appear slight but slowly growing signs of follicular stimulation. This new cycle, generally long, may again be amenorrheic, but is not always necessarily so.

For 6 to 10 days following the menstruation, the smears may still remain of luteal or pre-menstrual type; the follicular stimulation appears only late, for the growth of the new follicle is slow and retarded.

These cytological aspects may well arise from the inertia of the follicle (BONIME) - that is, from the presence of a follicle which

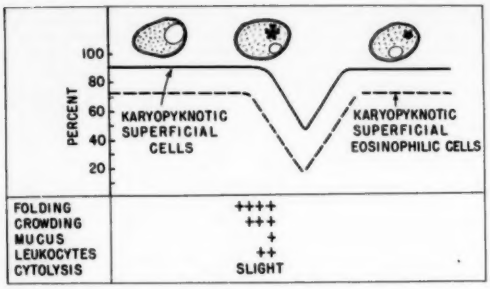

Fig. 1.

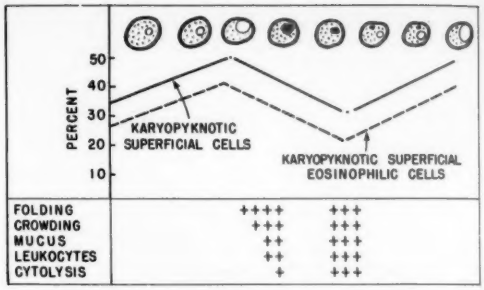

Fig. 2.

has reached maturity but regresses very slowly; though its functional activity is never very great, its disappearance is never complete. These are the follicles, erroneously qualified as "atresic" on the histological slides, which continue to secrete estrogens. Their added activity determines a cumulative effect on the endometrial receptor, which reflects the sum of the hormonal actions, whereas the vaginal receptor is only a mirror of a transitory functional "moment."

This, then, is one of the apparent discrepancies between biopsies and smears. Whereas the latter always show a follicular stimulation low or well below the normal, the endometrium, generally of slight thickness, shows the presence of rather numerous mitoses. Sometimes, though less often, it is hyperplastic. This hyperplasia is seen especially during the pre-menopause, a period during which the luteal phases are most often absent.

CONCLUSIONS

Although, generally speaking, the high level of pyknosis and of eosinophilia and the scantiness or absence of mucus, testify to the persistence of the follicle, there are cases in which this notion is no longer exact, and in which we must compare biopsies and several cycles of smears, in order to correctly interpret the follicular physiology. The concept of follicular "inertia" or persistence enables us to understand the apparent discrepancies of smears.

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HERBERT E. NIEBURGS
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To appraise the value of exfoliative cytology for the diagnosis of follicular persistency, consideration is given to feasibility of diagnosis, accuracy and limitations, as well as the clinical value of cytology in cases in which a diagnosis may be made in this manner.

In the adolescent and adult female of child-bearing age, the finding of a high Karyopyknotic Index in the vaginal smear taken during the two weeks prior to onset of the expected period, is suggestive of a non-ovulatory cycle. This diagnosis can be made provided there is absence of infection. Such evaluation is often useful in the study of infertility cases. The examination of a cervical smear taken simultaneously, but separately, may permit further evaluation as to the state of estrogenic function. While the presence of intermediate and superficial non-cornified cells is suggestive of a certain degree of estrogen deficiency, the presence of a high Karyopyknotic index in the cervical smear is also possible because of lack of ovulation in the preceding cycle.

With prolonged follicular persistence, the Karyopyknotic Index in the vaginal smear may decrease even to complete absence of karyopyknotic cells. This is often associated with marked desquamation, folding and crowding of cells, which may cause some difficulties in interpretation. Whether the cervical smear is of aid in such cases depends upon the duration of the follicular activity.

In cases of a missed period, the possibility of pregnancy may at times be ruled out by the detection of a high Karyopyknotic Index in the vaginal smear. In the differential diagnosis of amenorrhea, due to either follicular persistency, estrogen deficiency, or possibly early menopause, exfoliative cytology is frequently useful. According to published reports, and personal experience during the war in England, many women developed amenorrhea due to the emotional trauma of bombing. The endometrium in such women appeared to persist in that state of the cycle which prevailed at the time of the emotional shock. The vaginal smear findings in such cases were revealing.

In cases of vaginal bleeding, it is essential to rule out the possible presence of cervical or endometrial malignancy. Under such circumstances, the suggestive persistency of follicular activity by a high Karyopyknotic Index should be interpreted with caution, since an increase in the number of karyopyk-

notic cells has been observed with cervical and endometrial malignancy. On the other hand, the absence of cytologic evidence of follicular persistency may have diagnostic significance.

PETER STOLL
Heidelberg, Germany

Follicular persistency is the persistence of a group of follicles in the ovaries, including the persistence of the Graafian follicle if present. No ovulation takes place. The endometrium is subjected to an abnormally prolonged estrogenic action, which causes hyperplasia of the endometrium, but also sometimes hyperplasia of the mammary glands and special endocrine disturbances, which makes the patient feel uncomfortable. The close reciprocal relationship between the ovaries and the anterior lobe of the pituitary gland makes the disturbance a general one, especially if we consider the important role of the pituitary gland in the concert of endocrine glands.

Bleeding does not occur until:

a) withdrawal of the estrogenic hormone occurs (withdrawal-bleeding)
b) the hyperproliferation of the endometrium in regard to the estrogenic level present is too far advanced, and the nutrition of the hyperplastic tissue in circumscript areas is insufficient.
c) delayed ovulation with following lutein-production takes place (hyperplasia with secretory effect)
d) pressure of the highly proliferated endometrium in the uterine cavity develops superficial erosion and ulceration (necrobiosis of the endometrium, superficial).

The condition should be divided into two groups:

Short-term follicular persistency

Especially during the beginning and the end of normal ovarian function (prepubertic and climateric age)

Occasionally in the reproductive age (anovulatory-cycle)

Bleeding may occur at the time of the expected onset of menstruation, but the endometrium as well as the vaginal epithelium do not exhibit the effect of corpus luteum action (pseudo-menstruation)

Patient may not suspect any distrubance at all.

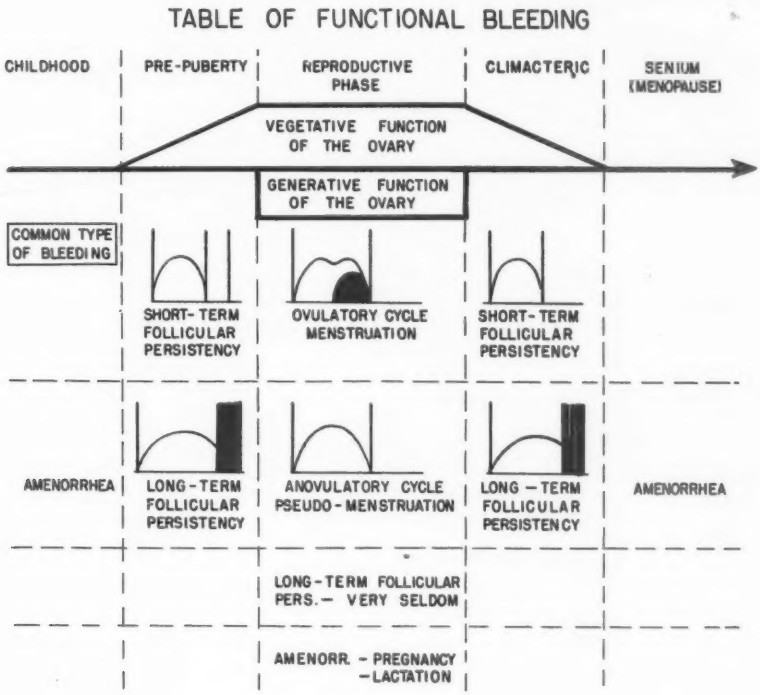

Fig. 1

Consulting the doctor only in case of sterility.

Long-term follicular persistency

Mostly normal in prepuberty and post-puberty, indicating the beginning and the end of regular ovarian endocrine activity.

Seldom in the reproductive age, but well known as "ghetto-amenorrhea" or "situation-amenorrhea," especially in women with unstable cycles.

Amehorrhea of several weeks or months is followed by prolonged and sometimes abundant bleeding and gives indication for D & C (Metrorrhagia, the old "metropathia haemorrhagica" of Robert Schroder)

Patient is suffering from bleeding and secondary anemia

Consulting doctor about bleeding

While long-term follicular persistency is of clinical interest and indicative for curretage with subsequent histological examination of the tissue, short-term follicular persistency will hardly be found, unless the patient is under close observation with temperatures, repeated vaginal smears and/or endometrial biopsies at the beginning of uterine bleeding. These three methods are very worthwhile in cases of sterility, to control the cyclic changes and to define clearly the type of cycle as ovulatory or anovulatory.

The frequency of short-term persistency during the reproductive time of life is, therefore, not exactly known. Defining one present cycle as anovulatory, we do not know whether this is only restricted to this special cycle and the following cycle will be ovulatory, or whether or not some more anovulatory cycles will follow. Only if the patient is cooperative and the methods mentioned above can be repeated over several months, will we obtain more information in each case. Ovulation may be prevented by some kind of physical or mental stress. We found, for example, in patients admitted to the hospital during the first 14 days of the cycle and operated upon some days later in the expected secretory stage, that 12% did not show secretory transformation of the endometrium and/or signs of secretory-phase within the vaginal smear (prevention of ovulation by hospitalization).

In our out-patient department we found, in otherwise healthy patients with normal 28 day cycles, in the second half of the cycle about 20% with an estrogenic-smear, indicating that ovulation probably did not occur. Most of these patients were workers (industrial-workers) or busy with some kind of work; they were not adapted very well by condition. Most exhibited signs of pelvic congestion (pelipathia), suffering with backache, headache,

TABLE I

Endometrial diagnosis		Vaginal smear			
		High proliferation (estrogenic)	Middle proliferation (luteal or androgenic)	Without proliferation (atrophic)	Insufficient material
Hyperplasia	207 (81,5%)	183 (72%)	19 (7.5%)	5 (2.0%)	-
Hyperplasia with partial secretory effect	17 (6.7%)	7 (2.8%)	10 (3.9%)	0 0	-
Without proliferation	25 (9,8%)	19 (7.4%)	3 (1.2%)	3 (1.2%)	-
Insufficient material	5 (2,0%)	0	0	0	5 (2.0%)
Totals	254 (100%)	209 (82.2%)	32 (12.6%)	8 (3.2%)	5 (2.0%)

COMPARATIVE STUDIES IN ENDOMETRIAL AND VAGINAL SMEARS IN CASES OF LONG-TERM FOLLICULAR PERSISTENCY WITH METRORRHAGIA (254 CASES).

migraine, constipation, restlessness and bad nervous condition.

When we find short-term follicular persistency by means of cytology this gives us an important clue to go further with the general examination and exploration in order to adjust the treatment to the special condition of this individual patient. We avoid hormones in these cases. Our prescription is leisure, recreation, pleasure and outdoor-sports, sometimes supported by belladonna or tranquilizers of some kind.

Short-time persistency of the follicle is part of manager-disease in women occurring in the childbearing age.

The frequency of long-time follicular persistency is better known, because the symptom - amenorrhea followed by bleeding - is very impressive to the patient. It can be divided in prepubertic and postpubertic. In those cases a dilatation and curettage usually is done by instruments, however, in recent years hormonal curetting by application of estrogen-lutein components is widely used. I personally would prefer the curettage by instruments with the following histological examination, which gives us a proper diagnosis and avoids overlooking malignancy, especially in elderly women. This gives us also a steady background for hormonal treatment.

Our comparative studies of patients with follicular persistency by means of histology and cytology include 254 patients, in whom we performed a curettage in order to stop bleeding. The cases are listed in Table I.

One sees immediately that there are some differences in histological and cytological findings. The reaction level and the reaction time of the endometrium and the vaginal wall to hormones is different. This gives us the possibility of defining very exactly the reason for bleeding, as is shown in Table II. The kind of treatment varies with the comparative findings.

After curettage and under the following hormonal treatment, if indicated, cytology plays the most important role in controlling the following course of endocrine function. Smears should be taken in 14 day intervals in order to avoid recurrence of the disease by application of corpus-luteum hormone.

TABLE II

	Histology	Cytology	Endocrine function of the ovary	Reason for bleeding
A	Large amount of tissue with typical hyperplasia	Marked estrogen effect	Follicular persistency (Present estrogenic level high enough to support hyperplastic endometrium)	Necrobiosis, localized
B	Small amount of tissue, without proliferation (functionalis already discharged)	Marked estrogen effect	Follicular persistency with decreasing estrogen level (Present estrogenic level not high enough to support hyperplastic endometrium)	Withdrawal-bleeding of long duration
C	Large amount of tissue, typical hyperplasia	Atrophic	Atresia of the follicles (Estrogen level dropped down very fast)	Acute withdrawal-bleeding of short duration
D	Large amount of tissue typical hyperplasia	Middle proliferation (luteal type)	Persistency followed by luteinization	Withdrawal bleeding with insufficient corpus luteum effect
E	Hyperplasia with localized secretory effect	Middle proliferation (luteal type)	Persistency followed by luteinization	Insufficient secretoric transformation of endometrium

TAKING THE FINDINGS OF HISTOLOGY AND CYTOLOGY INTO ACCOUNT, THE ABOVE DEFINITIVE DIAGNOSIS OF ENDOCRINE DISTURBANCE CAN BE GIVEN.

Dividing follicular persistency into the groups of Table II, one immediately has some clues in regard to hormonal therapy:

A. High dosage of corpus-luteum hormone, probably combined with androgenic hormone.
B. Corpus-luteum hormone combined with estrogen.
C. Follicular hormone, followed by corpus-luteum hormone.
D. Follicular hormone, combined with corpus-luteum hormone.
E. Corpus-luteum hormone.

DISCUSSION

ARTURO ANGEL ARRIGHI and GUILLERMO TERZANO, Buenos Aires, Argentina:

When ovulation does not occur and the follicle persists, serial vaginal smears reveal lack of progestational effect. In such anovulatory cycles the principal characteristics in the smears are absence of cellular clusters and absence of folding and curling of the cells (or these are very scanty and of no significance).

Two smears may be sufficient to make a diagnosis, but we do prefer serial smears. However, in 70 cases that we diagnosed as negative for progestational effect, 8 endometrial biopsies were positive, representing an error of 11.4%.

There are circumstances, adolescence for instance, when we have to depend on urinary smears. These are reliable also in diagnosing follicular persistency.

WERNER BICKENBACH and HANS-JÜRGEN SOOST, Munich, Germany:

The existence of follicular persistency can generally be concluded from the cytological picture only in connection with knowledge of the clinical findings of the exact cyclic history.

The vaginal smear with typical signs of estrogenic effect or of hyperfolliculinism (predominately karyopyknotic, eosinophilic and cyanophilic superficial cells and large intermediate cells which are flattened out and lying singly) is indeed typical for many cases of follicular persistency, but the question is not a specific cell picture. One can conclude a follicular persistency if this cell picture remains unchanged at least 18 to 20 days from the time of the last menstruation.

If a typical cell picture is not to be found, it does not say anything against follicular persistency. It may be the following:

1. If the effect of the follicular hormone exists long enough and is equally high and does not increase there will occur a diminishing of cell turgidity and slight decrease of Karyopyknotic and Eosinophilic Indices. In this manner a deficiency of hormones may be pretended. There is, in contrast to the picture of the luteal phase, less marked accumulation of cells.

2. Also at normal or even slightly decreased level of follicular hormone the picture of a glandular cystic hyperplasia of the endometrium can be developed, if the follicular hormone is effective long enough and without counter actions.

3. There is not an absolute deficiency of progesterone in all cases of follicular persistency. Sometimes sufficient progesterone formation is retarded. If then the sensitively reacting vaginal epithelium shows a typical picture of luteal phase, the dose is not enough to change the endometrium into a fully secretory phase.

4. If a persistent follicle becomes atretic, it may effect a so-called withdrawal bleeding. The vaginal smear then shows a distinct decrease of the effect of follicular hormone, perhaps down to the level of the atrophic cell picture. This change occurs generally very rapidly, but slows down when approaching the onset of menstruation.

(Conversely, the signs of high action of follicular hormone at the vaginal epithelium are often found while the curettage shows up only rests of endometrium which exfoliate).

The divergences between vaginal cell finding and histological endometrium finding, clearly schematized by Stoll and also demonstrated by de Brux, are therefore only apparent. They are to be explained by the different reactions of the organs for reception of hormonal stimulation. The vaginal epithelium is reacting by hormonal stimulations more sensitively and faster than the endometrial, and because of the steady exfoliating of the vaginal epithelium the cytological smear shows most clearly the hormonal situation of the body. In contrast to that, the endometrium, which lacks the steady cell exfoliation, reflects all hormonal stimulation which has been effective since the last bleeding. The cytological finding complementing the histological examinations always offers information about the present hormonal situation from which important points for therapy can be deduced.

We have reasons (explained previously), in contrast to Nieburgs, to think that the smears from the ectocervix are not suitable for judging the hormonal situation under suspicion of follicular persistency. We therefore take the smears from the lateral vaginal fornix.

Our system is as follows: at early bleedings we perform the hormonal therapy, if possible, under strict cytological control. This is nearly always successful with the intensely effective hormonal substances which are now available. For women of menopausal age with bleeding disturbances we think it useful to perform a curettage first for exclusion

of malignancy. The cytological finding still proves useful for control of further hormonal therapy. We especially appreciate, as does Stoll, the cytodiagnosis for prophylactic examination of patients who had a glandular-cystic hyperplasia. Relapses can be prevented by progesterone administration, if the cell picture of the increasing effect of follicular hormone continues to be the same without signs of influence of the corpus luteum hormone beyond the 18th to 20th day.

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UGO CITTI, Bologna, Italy:*

The systematic use of vaginal cytology in almost all of the endocrine diseases in the Department of Obstetrics and Gynecology of the University of Bologna during the past 10 years, has allowed us to arrive at certain considerations regarding the diagnostic possibilities of this method in cases of follicular persistency.

To begin with, we have been able, clinically, to observe that this pathological condition occurs with greater incidence during puberty and pre-menopause. In our statistics regarding the cases in which the diagnosis of follicular persistency was correct, 26% of the cases were observed in patients during the puberal period; 67% in patients in the pre-menopausal period; and 7% in patients during the reproductive age.

Therefore, it is clear, according to de Brux (1), that this is a pathology involving both extremities of the female genital cycle.

The diagnosis of this endocrinopathy is not always easy and requires systematic study for at least an entire cycle in cases of amenorrhea and several vaginal smears, or curettage followed by histological examination and cytology in cases in which metrorrhagia is present. Because this condition can be present in these two ways, we may distinguish, for purposes of study, those cases in which metrorrhagia is present from those cases with amenorrhea.

Most of our observations (about 80%) have been made on patients with metrorrhagia, following either a delay of the menstrual period or an amenorrhea for more than one month. Nevertheless, we cannot assume that metrorrhagia is a constant sign since it is obvious that this symptom is the only one which brings the patient to the physician, so that cases with amenorrhea are less often seen.

The cytological pictures during the period of hemorrhage were not always identical. In fact, they varied not only in regard to the Karyopyknotic and Eosinophilic Indices (even in those cases in which the metrorrhagia had been present for the same length of time) but also in the function of the metrorrhagic period. In those cases in which the smear was taken at the beginning of the metrorrhagia, 63% of them had moderate estrogenic stimulation, 15% had marked estrogenic stimulation, and 12% had mild or almost absent estrogenic stimulation. The histological report of the endometrial biopsy, at the beginning, showed an endometrial hyperplasia, in which 73% of the cases were of the follicular type and 27% of the cases of the mixed type.

These pictures were accompanied by a vaginal smear showing moderate follicular stimulation in the first case and progesterone effect in the second case. Therefore, a discrepancy is evident between the two tests in these cases, as also noted by other authors in cases of hyperfolliculism (2, 3, 4).

In those cases in which the metrorrhagia was already present for a certain length of time, the histological report showed an endometrial hyperplasia without follicular patterns in 50% of the cases. These histological patterns were accompanied by cytological patterns of the follicular type with moderate stimulation in the first case, with patterns due to luteal stimulation in the second, of the marked follicular type in the third, and with slight estrogenic stimulation in the fourth.

In those cases with amenorrhea, we have observed that the first smear showed mostly a cytological pattern due to a moderate follicular stimulation in 75%; in the remaining 25%, all forms of follicular stimulation. In this remaining 25%, all the transition forms were present from a marked follicular picture to a picture with slight estrogenic stimulus.

The histological picture of the endometrial biopsy has shown a correspondence with metrorrhagia.

We have performed vaginal cytology every other day for one month in the patients on whom we did not perform a curettage, because we did not consider a single examination able to give a diagnosis. The variations observed, have not generally been marked. In these cases we have observed, as de Brux (1), two different cytological pictures: a cytological pattern corresponding to a marked follicular stimulation and a cytological picture with a persisting moderate estrogenic stimulation. Because we have collected the samples in these cases for approximately one month, we have been able to follow the various evolutions of the cytological and clinical picture.

In 65% of the cases of the first type, after a length of time almost always longer than one month, a metrorrhagia appeared with an endometrial histological pattern referable to the hemorrhagic metropathy of Schroeder (5).

The vaginal cytological pictures in these cases fail to show significant variations and the Karyopyknotic and Eosinophilic Indices always were elevated. In the remaining 35%, at a certain point, one could see a slow decrease of the estrogenic stimulus, but without arriving at a luteal pattern and the appearance of metrorrhagia. The histological picture of the endometrial biopsy showed a mixed follicular-luteal hyperplasia. In the second type of vaginal cytological pattern the level of the Indices was rather low, and in 27% not only showed no variation, but metrorrhagia would not always appear

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and the amenorrhea was more frequent.

In 54% variations, but not always cyclic variations, were observed. These variations were characterized by a diminution of the Indices to very low values, by the appearance of some luteal features, but without the appearance, in most cases of menstruation. When menstruation would appear, the endometrial picture often showed hyperplasia without clear signs of proliferation.

The smear in these cases would show a slight or absent estrogenic stimulus.

In explanation of the discrepancy, we have referred to the diverse responses of the endometrium to a persistent stimulus which differs from the vaginal epithelium which expresses functional endocrine status. Even this fact necessitates the taking of specimens for vaginal cytology over a period of time.

From our experiences we can conclude that the interpretation of cytological findings alone is not sufficient to diagnose this condition. In fact, in cases of amenorrhea the persistence of high values of the Eosinophilic and Karyopyknotic Indices can without doubt indicate the diagnosis of follicular fluctuation with the appearance of metrorrhagia. In many cases this appears in the menstrual period, where there can occur findings to indicate a hematic loss of follicular persistency by true and proper menstruation followed by ovulation. We can obtain help in this by an endometrial biopsy when the uterine mucosa shows a folliculinic phase, for since there sometimes appears an initial luteinic aspect of the "irregular ripening" type of Traut and Kuder (6) and of Hamblen (7), one cannot always blame the follicular persistency (8).

In cases of active metrorrhagia the agreement of histological and cytological reports is the best means for arriving at a diagnosis. In these cases the interpretation can be difficult, since quite often there exists deceptors. However, if one takes samples from a few cycles a diagnosis can very often be made.

In conclusion, the vaginal smear is an invaluable aid in these cases provided that it is interpreted correctly. One must keep in mind the temporal changes that may occur.

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WOLFGANG KORTE, Bonn, Germany:

Follicular persistency, the cause of which is unknown, represents a morphologically demonstrable functional disorder of normal ovarian activity. Estradiol is, according to existing knowledge, the follicular hormone which is produced in the maturing follicle and especially in the Graafian follicle of the normally functioning ovary.

This follicular hormone fraction has well defined proliferative effects on the various tissues of the female genital organs. The qualitative comparability of this effect on the endometrium and the vaginal epithelium depends upon the cellular responsiveness of the different tissues and also upon the time factor, as Stoll has emphasized.

According to the more recent studies of Puck et al., one has to consider that not only estradiol but also estriol has a proliferative influence on the genital region. It is also known that estriol has a proliferative effect on the vaginal epithelium in particular. However, the stimulus on the endometrium seems to be insufficient for normal proliferation. Here there occurs pictures which are strikingly similar to the cystic form of the so-called glandular-cystic hyperplasia of the mucus membrane. From this one could conclude that the composition of the hormone, which is produced in the persistent follicle, is not identical with the contents of a normally mature follicle. In fact, one finds in cases of glandular-cystic hyperplasia of the mucus membrane, due to follicular persistency, considerably increased estriol in the blood and urine. It is understandable then that a discrepancy may occur between the histological proliferative picture of the endometrium and the cytological height of maturation of the vaginal epithelium. This should be studed methodically in order to determine whether or not a certain regularity is present.

I doubt if it is good to separate the term "follicular persistency" from the well defined disease picture of glandular-cystic hyperplasia of the endometrium (formerly metropathia hemorrhagica of Robert Schroder). I am speaking here of the anovulatory cycle. The follicle from which a mature ovum does not erupt does not persist in the literal sense. "Persistere" (Latin) means "to continue." One could say that the erupted follicle "continues" in another form. However, then it is called a corpus luteum. Persistency in the classical sense means continuation without follicular eruption beyond the period of normal follicle maturation. In the anovulatory cycle the follicle does not quite become mature. De Brux states: "One follicle reaches maturity, even though the ovum has already degenerated at the start, but it does not erupt, etc." I do not think that one should term such a follicle "mature." One cannot separate the maturity of the follicle from the term of the ovum which is intact and ready to erupt. A follicle, the ovum

of which has degenerated earlier or died, can be hormonally effective. It can cause genital bleeding and therefore is not a real menstruation.

Stoll has already pointed out the necessity of a curettage in cases of persistent bleeding due to glandular hyperplasia. Dilation is seldom necessary because the cervix is almost always soft, loose and wide. A hard uterine fundus is nearly a pathognomonic sign.

The term "medical curettage" is a "contradictio in adjectu." One should use this combination of words, if at all, only in quotation marks. We always perform the curettage as Stoll does. In young girls one can attempt to induce a shedding of the diseased endometrium by hormonal medication if the hyperplasia reoccurs after a short time. In the climacterium this becomes more dubious. Siebke observed a histologically proven case of glandular-cystic hyperplasia of the endometrium which was followed ten months later by bleeding due to an endometrial carcinoma. This bleeding was considered in another institution as a recurrence of the hyperplasia and was scheduled to be treated by hormones.

WARREN R. LANG, Philadelphia, Pennsylvania, U. S. A.:

If, as Nieburgs mentions, there is no vaginal inflammation, the vaginal smear is a fairly sensitive indicator of persistent estrogenic stimulation. Unfortunately, the smear tells us nothing of the reason for the prolonged estrogenic effect and, in actuality, the differential diagnosis revolves around the causes of hyperestrinism.

In the final analysis, follicular persistency must be a histologic diagnosis or at least one determined by direct ovarian observation. Follicular cystosis, single or multiple, is essentially a disturbance of physiology rather than true neoplastic change. If hyperestrinism is associated, the vaginal smear offers confirmatory evidence of the condition. In addition there are often irregular menses with a tendency to amenorrhea and possibly cystic ovaries by palpation. Laboratory studies show a lack of regressive change in the smear before onset of bleeding, lack of the characteristic cervical mucus cycling of the ovulating woman, the presence of an estrogenic endometrium and diminished urinary pregnandiol excretion.

LUIS MONTALVO RUIZ, Madrid, Spain:

Vaginal cytology study for the diagnosis of follicular persistency does not have absolute value, in as much as the persistently elevated Karyopyknotic Index during the second half of the cycle can go together with an endometrial secretory phase. This was mentioned before by Rockenshaub (2) and Polloson, Mattieu, and Haour (1) also found an elevated Karyopyknotic Index in both phases of the cycle. We agree with the authors in their fundamental principles, only we would like to emphasize the necessity of taking daily smears, and beginning with the 14th day of the cycle, if the Karyopyknotic and Eosinophilic Indices do not drop and the smears continue to contain turgescent, pink, superficial cells without leukocytes or mucus, follicular persistency must be supposed and a confirmatory biopsy must be performed on the 25th or 26th day of the cycle.

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OTAKAR NYKLÍČEK, Náchod, Czechoslovakia:

The value of exfoliative cytology in the diagnosis of follicular persistency is undisputable, but it is also of limited value. It is correct to also use further methods, such as biopsy of the endometrium, fern-phenomenon of the cervical mucus, and eventually even determination of the basal temperature for completing the diagnosis.

A cytological criterion of follicular persistency should theoretically find the Karyopyknotic Index of high value. However, in practice the evaluation is not as simple and the results are not definite.

Some time ago (1950-1953) we cytologically followed 141 women, in which a hyperplasia of the endometrium had been proven histologically. We took vaginal smears from them during the amenorrhea and also in the following period of bleeding. Thus, in the end we evaluated 612 vaginal smears.

We have classified each smear into one of the 3 so-called "estrogenic" types (hyperestrogenic, normoestrogenic, and hypoestrogenic, so that we might better compare the results (1)).

We have found that about two weeks before the bleeding begins, the proliferation of the vaginal mucosa reaches its highest point. Then the curve of hyperestric types moderately decreases. On the 4th to 7th day preceding the bleeding it drops more steeply and then still more steeply on the first day of bleeding. In the period of bleeding the curve of hyperestrogenic types descends substantially, even down to 32.2% in the women that have been bleeding a whole week.

These findings well support the opinions of the main speakers, especially those of de Brux.

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J. PAUL PUNDEL, Luxembourg, Luxembourg:

As the main factors of the etiological background of anovulatory cycles have been

presented so well by the main speakers, I will limit the discussion to only the cytological diagnosis of anovulatory cycles. I agree completely with the main speakers that the cytological diagnosis in these cases sometimes is very difficult. From my own material, I have gained the following conclusions:

1. The diagnosis of anovulatory cycles can be done only by repeated vaginal smears. A single vaginal smear taken during the premenstrual period from the 25th to the 28th day can lead to a false diagnosis of anovulatory cycle in over 25% of true and normal ovulatory cycles, if the progesterone effect disappears shortly before the onset of bleeding.

2. The cytological diagnosis of anovulatory cycles is the easiest in apparently normal cycles of 28-30 days duration. Here the smears show, in general, a rather strong estrogenic stimulation with mostly flat and isolated superficial cells. The so-called ovulatory peak either does not occur, or it is followed only by shrinking and folding of the cells, without grouping in clusters.

3. In clinically abnormal cycles, especially in cases of prolonged follicular persistency, the diagnosis by vaginal smears can be very difficult. Even if in these cases the endometrium can show at the beginning of bleeding an important endometrial hyperplasia, the vaginal smears frequently show during the entire period of amenorrhea only a moderate degree of estrogenic stimulation and in some cases the cells exhibit shortly before menstruation, pictures similar to progesteronic reactions or even to pregnancy (the so-called pseudopregnancy smears). The differential diagnosis between these smears and those of the normal postovulatory phase is sometimes very difficult. But if daily smears can be obtained at least two weeks before the onset of bleeding, ovulation can be excluded in most cases, since the progesterone-like pictures have not been preceded by typical ovulatory smears.

4. In cases of cytolytic smears, which are observed rather frequently in prolonged follicular persistency, no hormonal diagnosis is possible.

The vaginal smear, therefore, can expose one to a false diagnosis in anovulatory cycles in the same way as in ovulatory cycles. We now compare the vaginal smears with the endometrial biopsy, accepting the fact that in several cases the vaginal smears show pictures similar to those observed during the luteal phase of the cycle, while the endometrium remains in a proliferative phase. In most cases, serial smears allow one to avoid an erroneous conclusion, but there remain some cases in which a clear cytological diagnosis is not possible, even in the absence of cytolysis. As neither the vaginal smears nor the endometrial biopsy permit absolute conclusions concerning ovulation, we now have to find out which of these tests is correct. Small quantities of progesterone or progesterone-like substances could be secreted in the ovary by luteal degeneration of follicular cysts or even in the adrenal cortex. As it has been shown that the vaginal epithelium is more sensitive to progesterone than the endometrium, these small quantities of progesterone could influence only the vaginal epithelium. But in exceptional cases I have observed a rather normal vaginal cytology during the menstrual cycle, while the premenstrual endometrial biopsy showed only proliferation. One case could not be studied further. In the second case it could be proved during several cycles, by additional injections of progesterone, that the endometrium was temporarily (after abortion) refractory to progesterone. In the third case, operated on in the premenstrual phase for appendicitis, a normal corpus luteum was found and histologically proved. It seems, therefore, that if we have no explanation for some progesterone-like reactions in the vaginal smears of true anovulatory cycles, there exist exceptional cases where the endometrium does not react in a normal manner to progesterone.

MOJMIR SONEK, Brno, Czechoslovakia:

In accordance with the statements of the above authors we have found follicular persistency to be most frequent in puberty and menopause.

We have found, however, certain differences between the two above states. In 186 cases of pubertal secondary amenorrhea we observed in 38% of the cases hyperfollicular patterns indicating follicular persistency. Although the smear was hyperfollicular, the values of the Eosinophilic and Karyopyknotic Indices were not as high as in climacteric follicular persistency. In the pubertal states of follicular persistency the cells were less isolated, the eosinophilic granules were less marked and also the further course differed from the course of follicular persistency in the climacteric age.

The climacteric hyperfollicular amenorrhea was in most cases followed by bleeding after a decrease of the estrogen level, whereas in young girls pathologic bleeding took place only in one third of the cases. In the remaining cases, the amenorrheic period was followed by a short bleeding in the form of pseudomenstruation, with the possible repetitions and the restitution of the menstrual cycle within a short time. In a certain small percentage of cases the hyperfollicular amenorrhea changed into a hypofollicular one during a period of several months, going through a number of interstages characterized by cytolysis of varying degree. The hypofollicular amenorrhea was prolonged and very difficult to cure.

We believe that pubertal follicular persistency is more dynamic in its course than that in the menopausal age and can be representative of several hormonal conditions (from the typical anovulatory cycle to hyperfollicular amenorrhea, which can further change into hypofollicular states, etc.). The strict division of Stoll into the long-term and short-term follicular persistency is not always possible, in my opinion.

Vaginal cytology is especially valuable in the above hormonal disturbances in the pubertal age. The therapy can be well controlled by cytology. Treatment with high doses of progesterone (hormonal curettage) is very successful in the hyperfollicular state,

especially in the state with an initial drop of estrogen level. The normal cycle can be restored by repeated treatment for several months.

The condition of the so-called moderate follicular stimulation mentioned by de Brux is very interesting. We found that progesterone in high doses had a good effect in several cases of hypofollicular amenorrhea, especially that following a hyperfollicular period. We suppose that these cases could be regarded as so-called moderate follicular stimulation. We distinguish this condition from the real hypofollicular amenorrhea which takes place after a longer period of time and the treatment of which is very difficult.

In our opinion, the main role of vaginal cytology is as follows: By means of systematic cytological examinations treatment can be started in the favorable hyperfollicular stage and the long-term juvenile amenorrheas can be avoided.

CLOSING REMARKS

JEAN de BRUX:

To Citti: The discussion by Citti interested me greatly, and I congratulate him on his fine cytological and histological work. His discussion nevertheless calls forth several remarks:

1. Those cases in which the endometrium shows a folliculoluteinic response should be eliminated from discussion. The fact that ovulation has taken place eliminates the diagnosis of follicular persistence.

2. An endometrial sample taken during or after uterine bleeding is of value only if a shred of mucosa which has not undergone the desquamation is still encountered (in particular, in cases of endometrial hyperplasia anchored to the fundus by arteries and a conjunctivo-sclerous pedicle; we call this type "titularized hyperplasia"). Most often, the fragments come from the zone of regeneration and have an appearance of a quiescent endometrium, hence the impossibility, in these cases, of obtaining any information on the endometrial response to hormonal stimulations.

To Korte: I do not believe that "follicular persistence" should be integrated in the entity "glandular-cystic hyperplasia" of the endometrium. This dystrophy often occurs in women who ovulate, but in whom the hormonal ratio shows an unusual predominance of estrogens. I believe, furthermore, that we should clearly distinguish between the Graafian follicle and the ovum. Even though the latter is surrounded by the follicle, it is nevertheless simply one of the elements of the ensemble. The ovum may disappear but its "envelope" continues to exist; the follicle continues to have an increasing functional activity although the ovum has degenerated. Such is the classic process of the follicles which become progressively atresic during the course of a normal menstrual cycle, whereas only one goes on to rupture with evacuation of the ovum. In fact, in normal cycles one often sees in the histological sections of the ovary, the persistence of follicles, which by their size are near to maturation, but nevertheless without the ovum, the "privileged" follicle having already ruptured several hours earlier. I believe that the process of anovulatory cycles is exactly the same as the one I have just described, but here all the ova have degenerated.

To Pundel: I am in agreement with his entire discussion, and I was very interested by his hypothesis concerning the small quantities of progesterone or progestational substances secreted in the ovary by luteal degeneration of the follicular cysts. I can only, then, draw a comparison between this and the varying and rather bizarre images of luteinization, or the theca of the persisting follicles, found in the ovaries of the Stein-Leventhal syndrome. This poses very interesting histological and endocrinological problems to be probed.

PETER STOLL:

Korte's observation with Puck and others, that estriol and estradiol (two of the three components of follicular hormone) show different proliferative effectiveness to the endometrium and to the vaginal epithelium, needs further attention and investigation with endocrine analysis in addition to cytology, histology and the clinical course. Different quantitative composition of the follicular hormone in certain physiological disturbances could give us some explanation of the controversy of our findings. Korte says, estriol has a proliferative effect on vaginal wall in particular, but the stimulus seems to be insufficient to induce normal proliferation within the endometrium. This reacts with pictures similar to glandular cystic hyperplasia. Korte also finds in cases of glandular cystic hyperplasia considerably increased estriol in the blood and urine. In those cases we would have, cytologically, a high Karyopyknotic Index with very high proliferation of the vaginal epithelium and the typical swiss-cheese pattern within the endometrium. This would not explain, however, the observation of true hyperplasia of the endometrium combined with only a moderate degree of estrogenic stimulation within the smear or the absence of cytological proliferation at all, which was observed by other investigators such as Pundel, de Brux, Terzano and ourselves.

I entirely appreciate the opinion of Bickenbach and Soost, that a normal or even slightly increased level of follicular hormone develops the picture of glandular hyperplasia within the endometrium, if the follicular hormone is effective long enough. I would add, that also a slightly decreased level of follicular hormone, if it acts long enough, might be able to develop hyperplasia of the endometrium. But I cannot follow them completely in saying that the Karyopyknotic and Eosinophilic Indices decrease if the level of the follicular hormone is equally high over a long period and does not increase. I also think that the findings of Citti do not support this opinion. Also Nyklicek

states that the curve of hyperestric types moderately decrease after a peak, but this is followed by bleeding as is to be expected. Nyklicek definitely states: two weeks before the bleeding occurs, the proliferation of the vaginal mucosa reaches its highest point and then decreases moderately. On the fourth to seventh day preceding the bleeding it drops more steeply and then still more rapidly on the first day of bleeding. This is in close harmony to the findings of de Brux and our own observations.

I agree with Bickenbach and Soost in saying that there might not be an absolute deficiency of progesterone in all cases of follicular persistency. Also Pundel has the same opinion, when he says: small quantities of progesterone or progesterone-like substances could be secreted in the ovary by luteal degeneration of follicular cysts or even in the adrenal cortex. Pundel is entirely correct in his conclusion that the vaginal epithelium is more sensitive than the endometrium to those small quantities of progesterone. I am very pleased to hear that he also has seen a case with a normal corpus luteum in the premenstrual phase without any sign of secretory transformation of the endometrium and that he feels that exceptional cases exist where the endometrium does not react in a normal manner to progesterone. It might be of interest at this point to say that after inducing a "hormonal curettage" with a mixture of estrogens and progesterione and after curettage at the time of the onset of bleeding, I always found a characteristic endometrial pattern: the glands remain within the proliferating stage and the stroma shows secretory effect and sometimes decidual reaction. The vaginal smear in those cases varies, but shows mostly a mild estrogenic stimulation.

The statements on certain differences in follicular persistency in puberty and climacteric age, given by Sonek are very well defined. I acknowledge entirely their statement that "hyperfollicular amenorrhea can change into hypofollicular amenorrhea" in a small percentage of cases with further endocrine disturbance, the treatment of which is very difficult. This is correct for the pubertic phase, it is quite physiological in the climacteric woman when she enters the menopause.

I would like to present some axioms on menstruation and pseudomenstruation or on uterine bleeding in general:

One should always keep in mind that during an ovulatory cycle not only one follicle exists and is hormonally effective, but a multitude of follicles in both ovaries (I used to say: follicle-apparatus), one of whom reaches full maturity with eruption of the ovum at a given time (Graafian follicle). Only this one erupted follicle builds the corpus luteum menstruationis with production of progesterone. All the other follicles continue to produce follicular hormone. One or more of these may reach maturity and erupt an ovum at a later time (paracyclic ovulation). The menstrual bleeding occurs hormonally at the 28th day of the cycle and 14 days after the eruption of the first Graafian follicle. This menstrual bleeding is caused by a decrease of the level of the follicular hormone following the degeneration of the "follicle-apparatus" which begins very abruptly at about the 24th day of the cycle.

This is also true for the anovulatory cycle. When no follicle erupts an ovum and the presumptive Graafian follicle does not change into a corpus luteum, this follicle as well as the other follicles persist until the end of the period, at which time all follicles degenerate and bleeding occurs by withdrawal of follicular hormone as well as in the ovulatory cycle.

When bleeding occurs after 28 days, only histological examination of the endometrium and not the clinical aspect can differentiate between a normal menstruation (secretory transformation) and a pseudo-menstruation (proliferative or hyperproliferative endometrium). Basal temperature and daily smears can help to distinguish the normal (ovulatory, biphasic) cycle from the false (anovulatory, monophasic) cycle. The anovulatory cycle is a physiological event during the puberty phase and during the climacteric phase, i.e., during the onset and the end of ovarian activity. The anovulatory cycle is not quite physiological during the reproductive age, but occurs far more often than we know, following physical stress-situations or psychical trauma.

The anovulatory cycle can be named "short-term follicular persistency," which means that the "follicular apparatus" exists for a duration of 28 days without building a Graafian follicle. Then the "follicular apparatus" degenerates.

In the long-term follicular persistency, the follicular apparatus does not degenerate, no Graafian follicle is built. Bleeding occurs after a given time, longer than 28 days after last menstrual bleeding when (a) the persisting "follicular apparatus" degenerates, (b) the estrogenic production remains on the same level and is, therefore, after a given time, not high enough to avoid shrinkage of the hyperplastic stroma, stasis and necrosis, (c) ovulation occurs by stimulation from the pituitary gland.

I am fully aware that I have greatly simplified the problems which have not been entirely solved in spite of the extensive work of a generation of scientists, including men like Robert Schroder, Robert Meyer, J.E. Markee, H. Okkels, E.T. Engle, G.W. Corner, W.M. Allen and C. Kaufmann. Systematic cytological examination together with histological and endocrine studies promise to bring more light into this darkness.

VAGINAL CYTOLOGY IN HYSTERECTOMIZED PATIENTS WITH SPECIAL CONSIDERATION TO PRESENCE OR ABSENCE OF OVARIAN FUNCTION AFTER HYSTERECTOMY

PIERRE HAOUR
Lyon, France

The effect of hysterectomy is different whether the ovaries are removed or not, as can be demonstrated by vaginal smears and by other methods of hormonal evaluation.

1. In hysterectomized patients, with the presence of ovaries, as has already been noticed by others, repeared vaginal smears taken after surgery show:

Before menopause: a. no regression of the estrogenic stimulation.

b. no changes in the cyclic variations of exfoliated cells.

However, a more intense progestational effect has been observed after hysterectomy (1). A persistent action of the corpus luteum has been also reported experimentally in animals (2, 3, 4, 5).

After menopause: regression to atrophy does not occur earlier in hysterectomized than in normal patients.

However, one must notice that most of the time after hysterectomy, cells of the basal type are exfoliated from the vaginal scar and from local atrophic zones which are produced by changes in the vascularization. If the smear is taken at this site it may be falsely interpreted as atrophic.

In a small number of women (16%), functional troubles (of the castration type) will appear after hysterectomy. This is related to the degree of pelvic lesions (7, 8). These symptoms may be explained by injury to the vascular blood supply of the ovary. It did not seem to me that this group of patients showed any differences in their cytology as compared with patients free of symptoms. I will appreciate the opinion of the discussants on this particular point. There is no difference either in the cytologic pictures of patients with subtotal hysterectomy (without castration), and a cyclic mucus secretion can often be observed at the cervical os at ovulation time.

The vaginal cytology findings are in accordance with the studies of the urinary hormonal excretion; there is no increase in FSH excretion (8, 9), and the level of estrogens and progesterone is not modified (10). Basal temperature graphs show persistent cyclic variations (10).

2. In hysterectomized patients with bilateral ovariectomy, changes in the cytology are similar to the changes appearing after castration alone. The vaginal smears show suppression of cyclic variations and regression to the atrophic type of smear. One hundred women who were hysterectomized with ablation of the ovaries and lymphadenectomized for cancer of the uterus were studied by us:

a. 60 premenopausal patients had 25% atrophic smears,

b. 40 postmenopausal patients had 30% atrophic smears.

The time when atrophic smears can be obtained is different in pre- and postmenopausal patients. It depends also on individual factors and many patients retain the normal or intermediate smear pattern for many years after surgery (1).

These results are in accordance with the figures observed in non-cancerous, ovariectomized patients without hysterectomy.

In conclusion, studies of vaginal cy-

tology show that the presence or absence of the uterus does not cause any significant modification in the hormonal status.

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B. CORNELIS HOPMAN *

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The vaginal epithelium of women responds to hormonal stimulation and as such reflects the hormonal status. The difference in the cell population of the vaginal smear before and after ovulation, as well as the changes which appear in the menopause, demonstrate this sensitivity. However, the general picture is not a regular or predictable one. One can find in postmenopausal women a vaginal smear which displays advanced atrophy with many basal and parabasal cells on the slides. In other cases the atrophy is much milder: intermediate cells dominate the vaginal picture. In still other cases a follicular type of smear may be present, even in old age. In general, however, one sees in postmenopausal women an atrophy increasing with age. That some hormonal stimulation still exists in the menopause is also evident from some observations made by Papanicolaou who described a sexual rhythm in postmenopausal women (1). The extra genital source of the hormonal stimulation probably is the adrenal cortex which shows hyperplasia in mice after gonadectomy (2). Desoxycorticosterone is able to produce growth of mammary glands and uterine bleeding in castrated monkeys (3).

To study the influence of uterus extirpation on the vaginal smear as related to ovarian stimulation, fifty cases were studied before and after this operation was performed. The results are not always reliable. Much depends upon the diagnosis before the operation, whether the ovarian stimulation was already high, as for example in hyperplasia mucosae uteri, in carcinoma, in some fibroids and in infected tissue as in pelvic inflammatory disease, or whether the ovarian stimulation was low, as for example in older women operated on for uterine prolapse. Superficial squamous cells, intermediate cells and basal cells were studied, counted and tabulated, The results were surprising in so far as the vaginal smear did not show an extensive change in one direction or another. Even when the ovaries were removed, together with the uterus, there was an atrophic cell picture in only 20% of the patients. In some of these patients there was already some atrophy before surgery. The change most often found is a cleansing of the vaginal smear picture because cervicitis, so often the cause of inflammatory products such as leukocytes, bacteria, mucus, etc., is removed with the uterus. At the same time the alkaline pH, prevailing when cervicitis is present, is changed to a slightly acidic pH changing the bacterial flora of the vagina from cocci and mixed bacteria to Döderlein bacilli and other lactic acid producing bacilli. Instead of the vaginal epithelia being regularly and evenly spread, as found in most slides before surgery, after hysterectomy we often found the cells united in large groups and separated by open spaces. In cases where the function of the ovaries is maintained, pyknosis and eosinophilia, criteria for ovarian stimulation, still exist. A few times it has even increased. Where the function of the ovaries has suffered, the cells look less mature, but atrophy is not seen. Decrease of ovarian function may be caused by loss of vascularization due to clamping of the arteries during surgery, pressure of operation stumps or operation on the ovaries themselves due to inflammation, cystic degeneration, tumor formation, etc. In some cases the vaginal smear may assume the characteristics of pregnancy. The cells show a dark border as do the Papanicolaou "oyster shell" cells. Protoplasm fragments, naked nuclei and Döderlein bacilli are present. The cells look more darkly stained than normal. All these signs resemble pregnancy (4). The cells, however, are too large for pregnancy and the typical stretched nuclei with chromatin condensations are absent or indistinct. The cause of these pregnancy changes is not known. It may be due to a relative decrease of ovarian function with less estrogen formation, or it may be caused by the formation of a corpus luteum persistency in the ovaries (5). In accordance with these facts is the cessation of rhythmic changes in the cell population of the vaginal smear sometimes encountered after uterus extirpation. However, the change to a pregnancy picture in the vaginal smear may also be observed after total extirpation of uterus and ovaries.

In general, it may be stated that in most of the cases, especially when the uterus extirpation is done for carcinoma in situ of the cervix or chronic inflammatory disease, there is a slight decrease in the ovarian stimulation (90% of the cases): the pyknotic eosinophilic cells existing before the operation (Fig. 1) are changed to the more cyanophilic vesicular type of squamous cells as found in less intensive ovarian stimulation. The cells may show characteristics similar to pregnancy (Fig. 2). In these cases we must consider the fact that cervical carcinoma is often accompanied by extensive infection of the cervix. Estrogen is concentrated in infected tissue (6) so that the change of pyknotic to vesicular nuclei after hysterectomy may be caused by the cleansing of the vagina rather than a decrease of ovarian stimulation. This possibility must especially be taken into account because of the fact that af-

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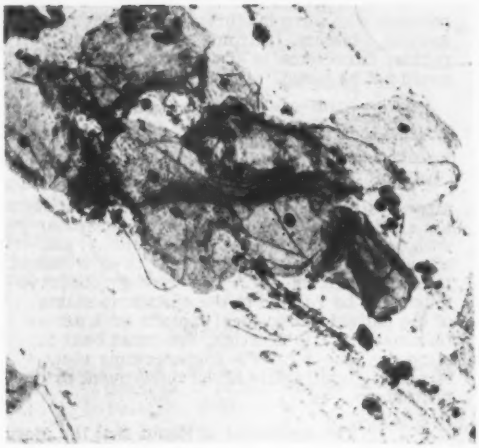

Fig. 1. Cervical smear of patient with carcinoma in situ. The normal squamous epithelia cells show a strong ovarian stimulation: most of them are eosinophilic and have pyknotic nuclei. There is much infection: many leukocytes, mucus and bacteria. (Only a clear portion of the slide is photographed to show the cells).

Fig. 2. Cervical smear of the same patient some months after hysterectomy. Normal squamous epithelia show a decreased ovarian stimulation: most of the cells are cyanophilic, and show vesicular nuclei. Here and there the cells take the shape of the Papanicolaou "oyster shell" cells with heavy stained thickened borders. Further pregnancy signs are naked nuclei (upper left and lower right) and protoplasm fragments. Bacteria and cocci are replaced by Döderlein and other lactic acid forming bacilli. The infection is considerably less than in Fig. 1.

ter hysterectomy for metrorrhagia or fibroids, where the infection before the operation plays only a minor role, the slides mostly show no change (or only slight change) after surgery. Furthermore, it has been found that when the infection persists after the operation in carcinoma in situ patients no decrease in ovarian stimulation takes place: the cells maintain their eosinophilic pyknotic characteristics. It is believed that these last cases indicate a less favorable prognosis.

I have no explanation for the increase of ovarian stimulation as seen in a very few cases after uterus extirpation. The idea that after uterus extirpation the estrogen present serves fewer organs and as such works more intensely seems too simple to be true.

SUMMARY

Uterus extirpation does not change ovarian stimulation in an extensive way as observed in the vaginal smear. In most cases a cleansing of the vaginal smear picture is observed together with a change of the vaginal bacterial flora of cocci and mixed bacteria to Döderlein and other lactic acid forming bacilli. Atrophy is not observed. Even when the ovaries also are removed, together with the uterus, atrophy is seen in only 20% of the cases. A mild decrease in ovarian stimulation is generally observed (90%). This change was more common when the operation was performed for carcinoma in situ and chronic inflammatory disease than for fibroids or metrorrhagia. Now and then a cytologic picture resembling that of pregnancy was seen. In a very few cases a mild increase in ovarian stimulation is observed. The causes for the changes are described as far as understood.

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ERICA WACHTEL
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Twenty-three women between the ages of 32 and 41 were studied cytologically for at least three months following total hysterectomy with preservation of one or both ovaries. Smears were taken at least once a week. Of these 23 patients, 4, or 17.4%, failed to show cyclical variations in their smears, whereas the remaining 19 women (82.6%) appeared to

have normal cycles. The smear patterns in these women, however, differed from those of untreated patients in so far as films taken during the secretory phase failed to show the influx of leukocytes normally occurring simultaneously with the clustering of cells and envelope formation. It is possible that the increase in leukocytes during the secretory phase in the intact woman is due to a cervical factor and that, conversely, the lack of leukocytes in hysterectomized patients may be explained by the absence of the cervix. In other respects the smear patterns were indistinguishable from those obtained from women with normal genital tracts.

Although the number of women studied up to now is still very small we conclude that hysterectomy, as a rule, does not interfere with ovarian function and that, where ovulation had been normal before operation, it continues normally at least for the next 3 post operative months.

DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

I agree with the three authors that hysterectomy, subtotal or total, has no appreciable effect upon the functioning of the remaining ovaries as evidenced by the vaginal smear. I also observed the "cleansing effect" of hysterectomy as described by Hopman, but only in cases of total, not subtotal, hysterectomy.

HANNES KREMER, Vienna, Austria:

We principally distinguish between two groups of hysterectomized patients. The first group includes hysterectomized patients whose ovaries were also removed. The second group includes patients where a simple hysterectomy has been done.

The first group: Besides intermediate cells, parabasal and basal cells were found in the smear of these patients. In some smears only some superficial cells were noticed. The question of how they were produced has not yet been solved (Adrenal glands?).

The second group: While screening the smears of this group of patients, we found cells from all layers of the squamous epithelium. Naturally this group includes patients who were menstruating at the time of hysterectomy.

May I add to these two groups of patients a third one: We implanted endometrium of the corpus of the uterus into the vagina of young patients in order to keep them menstruating, for psychological reasons. Also here, no difference could be found between these patients and those on whom a simple hysterectomy was performed without an implantation of endometrium into the vagina.

A special difference in the smears of patients who were hysterectomized because of varying reasons, (fibroma of the uterus, metropathia, carcinoma in situ, pyosalpinx, etc.) could not be found.

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

A discussion of vaginal cytology after hysterectomy should preferably include the changes after supravaginal hysterectomy, with or without bilateral oophorectomy; and after complete hysterectomy, with or without bilateral oophorectomy. The cytology observed must also be related to the endocrine status of the patient and to local factors such as inflammations. In addition, one must bear in mind the reason for the hysterectomy since this may significantly affect subsequent cytologic findings.

The statement of Haour that the uterus itself has no direct effect on the smear, except by the influx of cervical and to a less extent of endometrial elements, is probably quite generally accepted. Nor, as Hopman correctly indicates, are the changes regular or predictable after hysterectomy with or without removal of both ovaries. The observation by Wachtel that the presence of the cervix is associated with more leukocytes in the smear during the secretory phase than when the cervix is absent is an interesting one that certainly deserves more study.

HANS MUTH, Müenster in Westfalen, Germany:

In agreement with the studies of Hopman, Haour and Erica Wachtel, we too have not been able, with the aid of a large amount of cytological material, to affirm the assumption of an endocrine function of the uterus.

After hysterectomy, without removal of one or both ovaries in women in the reproductive age up to 42 years, we found in the vast majority of the cases a biphasic ovarian cycle in the vaginal smear.

After total hysterectomy, however, we found in one-third of the cases an ovarian insufficiency, as compared to 15.3% after a supravaginal amputation of the uterus. As a cause for these ovarian functional disturbances we consider postoperative changes in the vascular support of the organ.

The higher degree of cleanliness of the vaginal smear after hysterectomy, as reported by Hopman, may well be traced back to the elimination of cervicitis existing prior to the operation.

VIOLETTE M. NUOVO, Paris, France:

We studied cases of women during the reproductive age who had a hysterectomy and no ovariectomy. Our results are compatible with those obtained by Haour, Hofman and Erica Wachtel.

Whether the cervix was removed or not, the smears showed a slight decrease of pyknosis except for one patient. Therefore, the cervical factor does not seem of importance.

On the other hand, we found cytologic ovulatory changes in only one-third of the patients, although seven to eight smears were obtained from each patient (one every three days) and even though an ovulatory change was noticed on the temperature chart. Hormonal levels were performed on 13 of these patients and showed a normal level of estrone-estradiol, but no estriol. This seems to prove the part the uterus has in the metabolism of endogenous estrogens (1).

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EDMUND SCHÜLLER, Vienna, Austria:

I have also observed that the presence or absence of the uterus does not cause a modification in the function of the remaining ovaries. In 33 young women, 1 to 5 years following total hysterectomy with preservation of one or both ovaries, I controlled the function of the ovaries for 3 months. During the time of observation vaginal smears were taken every 5 days and the body temperature was recorded daily. In 18 women, smears and body temperature curve showed cyclical variations. The remaining 15 women who failed to show normal cycles had had no regular function of the ovaries before the hysterectomy.

CLOSING REMARKS

PIERRE HAOUR:

From the above discussions it appears that after hysterectomy with preservation of ovarian function there are no significant changes in cytology. However, different facts must be carefully considered in studying this problem:

1. The hormonal status of the patients before surgery must be investigated, if possible. The persistence or absence of cyclic variations after hysterectomy probably depends upon this status. This problem deserves more study.

2. The type of interference is also important. The presence or absence of leukocytes and of inflammation on the smears might be correlated to the presence or absence of the cervical portion of the uterus.

3. Depending on the vascular status after hysterectomy, cellular changes noticed in the smears may be falsely interpreted as estrogen deficiency. Care should be taken to perform vaginal smears on the wall of the vagina instead of at the vaginal pool: urinary smears can produce sometimes better results.

B. CORNELIS HOPMAN:

In answer to von Haam: I agree with von Haam that the "cleansing effect" after hysterectomy is only present in cases of total, not subtotal, hysterectomy. In subtotal hysterectomy the cervical stump is left behind, causing continuing cervicitis symptoms visible on the cytologic preparations.

In answer to Kremer: Kremer's point is an interesting one: To implant endometrium of the corpus uteri into the vagina of young patients to keep them menstruating. I found a difference in the smears of patients who were hysterectomized whether or not a cervicitis was present. In cancer where a cervicitis is very common, I found a cleansing of the smear accompanied by reduction of the estrogen effect. In cases where a cervicitis had not been present, usually no difference in the smears was found, before or after hysterectomy.

In answer to Lang: I agree with Lang that there is a difference in the vaginal cytology if the hysterectomy performed is total or if only supravaginal. As stated before, the removal of the cervix also eliminates the cervicitis (an important source of infection) in our cytologic preparations. I believe that the observation by Erica Wachtel, that the secretory phase is associated with more leukocytes is also caused by the "cleansing" effect of estrogen present in the proliferative phase, decreasing in the secretory phase.

In answer to Muth: I agree with Muth that ovarian insufficiency after hysterectomy may probably be caused by clamping of the ovarian arteries during the operation.

THE VALUE OF EXFOLIATIVE CYTOLOGY IN THE DIAGNOSIS OF HORMONE-PRODUCING TUMORS

JEAN A. de BRUX
Paris, France

In certain cases, cytology is one of the simplest, most rapid and most economical means of diagnosing hormone-producing ovarian tumors. Unfortunately, the complexity of the hormones secreted by certain of such tumors interferes with the growth and exfoliation of the vaginal mucosa, thus, sometimes rendering an exact diagnosis difficult, if not impossible.

Furthermore, not only tumors are involved here: for certain gonadal dysgeneses also determine cytological modifications suggestive of tumors (hyperplasia of the cortical stroma).

To understand the complexity of this question, it is necessary to know the histogenesis of the ovarian tumors having an endocrine action. Nevertheless, it could be supposed that the hormone-producing tumors originate from undifferentiated germinal cells having remained quiescent, and giving rise, at some moment, either to:

a. female elements - granulosa cells, thecal cells, or
b. male elements - sertoli cells, Leydig cells, or
c. a mixture of the two varieties of cells.

These tumors may occur in pre-pubescent girls, in young women, and after menopause.

The tumors most frequently encountered are:

1. Tumors of granulosa and thecal cells (granulosa-thecal-cell tumor),
2. Tumors of Sertoli and Leydig cells (arrhenoblastoma) and
3. Tumors of mixed cells (gynadroblastomas).

1. GRANULOSA OR THECAL CELL TUMORS

These generally have a feminizing action: precocious puberty, or post-menopausal metrorrhagias, and an increased estrogen level is found in the blood and urine. Cytology usually affords the diagnosis.

A. In the young girl presenting a very precocious puberty, there is a strongly increased level (90-95%) of superficial cells with pyknotic and eosinophilic nuclei. However, this same picture may also be found in cases of precocious puberty which are either of physiological origin or secondary to a cerebral tumor (pinealoma) or testifying to a general dystrophy (Albright's disease); these causes, consequently, should be eliminated in making the diagnosis of feminizing ovarian tumor.

B. In post-menopause likewise, other possible diagnoses must be ruled out before concluding that a feminizing tumor exists:

1. Unrecognized absorption of estrogens.
2. Possibility of an endometrial cancer (bearing in mind that both tumors may coexist; in this connection, the discovery of an active endometrial hyperplasia should be considered as evidence in favor of a feminizing tumor).
3. The hypertrophy of the ovarian cortical stroma sometimes seen in menopausal women may be an element of endometrial reactivation which does not necessarily indicate the existence of a tumor.

Unfortunately, there are also some cases where granulosa or thecal cells tumors are not accompanied by a syndrome of feminization, but on the contrary, produce a syndrome of masculinization, with hirsutism, acne, and markedly androgenic-type smears.

2. SERTOLI AND LEYDIG CELL TUMORS

These occur in young women (30 years, approximately), and are generally accompanied by a syndrome of defeminization, with enlargement of the clitoris, hirsutism, acne, modification of the voice, and amenorrhea. There is also found a slight rise in the 17-ketosteroids.

Here, the smears are decidedly androgenic, showing intermediate-type cells with pale but eccentric nuclei and a vitreous cytoplasm. However, just as there exist granulosa and thecal cell tumors having a paradoxical masculinizing action, similarly there exist Sertoli and Leydig cell tumors having a paradoxical feminizing action, in which the smears have a markedly increased level of superficial cells with pyknotic and eosinophilic nuclei.

3. MIXED-CELL TUMORS

But there also exist "mixed-cell" tumors excreting increased quantities of estrogens, or progesterone and of 17-ketosteroids. Histologically, it is often difficult to determine which of the cellular elements produce these different hormones, since it has been demonstrated that certain testicular elements may be feminizing, and certain ovarian elements masculinizing. Moreover, under certain conditions the same cells may alternately produce androgens and estrogens.

Those cases (2) in which we were able to examine smears were of the androgenic type, and the tumors were accompanied by a syndrome of masculinization.

We shall not discuss here the lipoid-cell tumors, whose histogenesis is practically unknown, whose effects are variable, ranging from Cushing's syndrome to masculinization, and whose hormonal excretion is complex and, above all, little known.

CONCLUSION

Cytology is of great value in matters of hormone-producing tumors: (a) when the excretion is clearly determines: estrogens or androgens; (b) when these tumors occur in patients not having, or no longer having, spontaneous hormonal secretions; (c) when the hormone is excreted in such quantity as to nullify the effects of the other secretions, and (d) when accessory factors capable of modifying the cytology have been ruled out (e.g., absorption of estrogens).

In an appreciable number of cases, however, the interpretation of the vaginal cytology in tumors of endocrine action is rendered delicate by the complexity of the secretions, but it is, nevertheless, often a valuable aid in the diagnosis.

MARCEL GAUDEFROY

Lille, Nord, France

I have observed a case of granulosa tumor, the diagnosis of which was made by abnormally high estrogenic vaginal smears. The patient was a 66 year old woman who presented slight metrorrhagia without pain or physical trouble: Karyopyknotic Index 68; Eosinophilic Index 73; no malignant cells in the vaginal, cervical or endometrial smears; glandulocystic hyperplasia of the endometrium; normal hysterographic pattern of the uterus and tubes. No hormonal dosages were made. The diagnosis was granulosa cell tumor of the ovary. Hysterectomy revealed no visible lesion or tumor of the ovaries. However, pathological study showed in the apparently normal left ovary a small granulosa-thecal mixed cell type tumor.

In my opinion, the occurrence of a durable estrogenic type in the vaginal smears after the normal menopausal time is a good indication of estrogenic tumor of the ovary (if the woman has not received estrogenic drugs!).

I have had no experience with vaginal smears in arrhenoblastoma of the ovaries, which should exhibit androgenic cell type.

EDMUND SCHÜLLER

Vienna, Austria

In the case of hormone-producing tumors, exfoliative cytology is of value only in the diagnosis of feminizing tumors of women and children. If there is an unusually high Karyopyknotic Index in the presence of an ovarian tumor, there is suspicion of a feminizing tumor.

During the last two years we observed seven feminizing tumors of women, between 56 and 70 years of age. In every case the Karyopyknotic Index was unusually high. In three cases the correct diagnosis was found by smear before the operation in connection with palpation of a tumor of the ovary. These cases were found by routine cytological examination for cancer detection. In the other four patients, the suspicion of a feminizing tumor was present from the beginning, due to uterine bleeding.

DISCUSSION

ABRAHAM RAKOFF, Philadelphia, Pennsylvania, U.S.A.:

Although the papers by de Brux, Gaudefroy, and Schuller properly point out the value cytological smears may have in arousing suspicion of a hormone-producing tumor of the ovary, I think the case has been somewhat over-stated and the limitations underestimated.

A highly estrogenic smear in the female child confirms the clinical impression of feminizing precocious puberty but does not make the differential diagnosis between syndromes of true precocious puberty and femini-

zing tumors of the ovary. In the post-menopausal woman likewise, as de Brux points out, there are benign conditions of the ovary which can result in hyperestrogenism.

Similarly in androgen-producing tumors of the ovary the vaginal smear is not at all specific, and depends upon the relative amount of androgen (and estrogen and other steroids) which are secreted. The so-called "androgenic" type smear may occur in adrenal hyperfunction, sometimes in ovarian hyperthecosis or diffuse luteinization of the ovaries, and sometimes even in ovarian deficiency not associated with increased androgen secretion.

ERICA G. WACHTEL, London, England, U.K.:

It appears from the statements of the main speakers that a high estrogenic inference in vaginal smears may indicate the presence of a feminizing ovarian tumor. However, the operative word is "may" and the cytologist, when faced with such a smear from an elderly woman with a history of postmenopausal or metrorrhagic bleeding, will not be in a position to present the gynecologist with a definite diagnosis of hormone-producing tumor. The most he can do is offer the suggestion of a feminizing tumor among all the other possibilities which may account for a high estrogen level in the smears. When faced with a direct question from the gynecologists as to whether or not the smear pattern is diagnostic of a hormone-producing tumor, the cytologist will not be of great help, since he will have to revert to a diplomatic "maybe," however cleverly his answer may be disguised.

With regard to androgen-producing tumors we have decided in a previous number of the ACTA CYTOLOGICA (Vol. I, No. I) that the androgenic smear type is not as definite as the estrogenic pattern. We are, therefore, again reduced to a suggestion of "compatible with" without really convincing proof.

In summary, at the present stage of our knowledge, I beg to disagree with de Brux's introductory remarks that "cytology in certain cases, is the simplest, most rapid and most economical means of diagnosing hormone-producing tumors." As he says himself, all the other causes of excessive hormone production have to be excluded first, which is neither simple, nor rapid and economical. Furthermore, it must be borne in mind that a high estrogen level in vaginal smears may be the only cytological indication of the presence of an ovarian carcinoma.

CLOSING REMARKS

JEAN de BRUX:

I fear that my thoughts have been somewhat exaggerated by the discussants. It is far from my idea that the vaginal smear could have any absolute value in the diagnosis of an ovarian tumor with endocrine action.

It is only an excellent indication of the appearance of a hormonal stimulation abnormal in point of time. It is a sign which should stimulate a more precise search (and which is sometimes the means of discovering an ovarian carcinoma which histologically may be banal but of which one is not certain that it is not hormone-secreting).

Sometimes the hormonal characteristics of the vaginal smear are of interest, particularly in certain arrhenoblastomas - but not in all - in which there appear so-called androgenic cells. As a matter of fact, this question is a very difficult one, especially in pre-puberty and in the menopause.

The ovarian tumors of endocrine action rarely secrete a single, pure hormone. Certain Leydig-cell ovarian tumors produce not androgens, but estrogens, as is proved by the presence of a gynecomastia in males having Leydig-cell testicular tumors.

Finally, the gynandroblastomas (term used by Robert Meyer) characterize tumors in which there are noted not only undifferentiated cells with presence of Call-Exner bodies, but also Sertoli and Leydig cells. These tumors, then, secrete various hormones - estrogenic, androgenic and progestational substances. We may imagine that in these endocrine cells the hormone production is abnormal and that the vaginal smear only reflects the algebraic sum of hormones of diverse and probably antagonistic activities.

Hence, I can only join in the diplomatic "maybe" of Erica Wachtel, but I persist in believing that in certain cases the vaginal smear is a simple, rapid and relatively economical means of approaching the diagnosis.

MARCEL GAUDEFROY:

I agree entirely with the remarks of Rakoff and Erica Wachtel. The highly estrogenic smear has great value in the diagnosis of estrogen-producing tumors, especially in young girls and old women, that is, before puberty and after menopause. However, their value is only in raising suspicion. Abnormal estrogenic smears must lead one to a complete investigation of the case in order to discover the true etiology of the estrogenic secretion, which often is a thecal ovarian tumor.

EDMUND SCHÜLLER:

The objections of Erica Wachtel and Rakoff are valid. I, also, am not of the opintion that a hormone-producing tumor can be diagnosed with only an approximate degree of certainty by means of cytology. In postmenopausal patients showing an unusually marked estrogenic effect the cause in most cases is of a different nature than that due to hormone-producing tumors, or, in some cases, cannot be found at all. However, encountering such smear patterns in children and postmenopausal patients, one should alert the clinician and point to the possibility of the presence of an ovarian tumor. Off and on, such a report may induce another thorough examination which then may lead to the detection of such a tumor.

THE LEVEL OF ENDOGENOUS ESTROGENS IN PATIENTS WITH CERVICAL CARCINOMA AS DEMONSTRATED BY METHODS OTHER THAN EXFOLIATIVE CYTOLOGY

JULES-ANDRÉ BRET and
FERNAND COUPEZ
Paris, France

The level of estrogens, chemically assayed, seemed to us an important factor in seeking the status able to influence the genesis of cancer or to go together with its clinical symptom.

Our actual research, still in progress, does not permit a definite conclusion, but seems to demonstrate the interest of such a subject.

We successively used the phosphotungesto chlorhydric hydrolysis, then the enzymatic hydrolysis with Jayle's technique applied to Kober's reaction. We found a high percentage of phenolsteroids increased in the cervical carcinomas.

Women during reproductive age

1. Acid hydrolysis
   levels considered as normal: less than 90
   total number of tests: 65
   values above 90: 42 (64%)

2. Enzymatic hydrolysis
   level considered as normal: less than 50
   total number of tests: 35
   values above 50: 21 (60%)

Menopausal women

1. Acid hydrolysis
   level considered as normal: less than 10
   total number of tests: 30
   values above 10: 29 (96%)

2. Enzymatic hydrolysis
   level considered as normal: less than 10
   total number of tests: 40
   values above 10: 40 (100%)

We cannot give more details here, but we would like to make three remarks:

1. The technique of extraction scarcely changes the results.

2. The folliculin complex and its urinary metabolites seem undoubtedly increased in numerous cervical carcinoma.

3. The menopausal period makes the increase more obvious.

DISCUSSION

HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D.F., Mexico:

We have found Bret's results obtained by chemical evaluations very interesting. We believe that these techniques are sufficiently safe for the demonstration of the urinary metabolites of the estrogenic compounds. We have no experience with the chemical methods, only with the biological methods which, to our understanding, are specific for the demonstration of the biologically active estrogens.

In one small number of patients with carcinoma of the cervix, we have made determinations of hypophyseal gonadotropins. The data obtained would indicate indirectly the level of endogenous estrogens.

We have reviewed the amounts of

follicle stimulating hormone (FSH) in women with carcinoma of the cervix and a comparison with the groups of controls was established in similar conditions, in age, menopause and moment in the menstrual cycle.

In the small number of cases studied until now, we have found the following:

Follicle Stimulating Hormone	Normal women (menstruating)	Women with uterine cancer (menstruating)
+55u.r.	11 (18.9%)	3 (16.6%)
6-40u.r.	15 (25.8%)	5 (27.7%)
-6u.r.	32 (55.1%)	10 (55.5%)
	58	18
	Normal menopausal women	Menopausal women with uterine cancer
+55u.r.	14 (60.8%)	12 (52.1%)
6-40u.r.	5 (21.7%)	7 (30.4%)
-6u.r.	4 (17.3%)	4 (17.3%)
	23	23

In spite of the fact that the small number of cases studied does not allow any conclusion, we can observe the following tendencies:

1. Practically no difference can be seen in the menstruating women.

2. In the menopausal patients with uterine cancer, there is a tendency to show a persistent estrogenic activity, slightly greater than that which is present in the patients without cancer. Our results in the menopausal women with cancer can be considered similar to those found by the main speaker, if we suppose that the estrogen level indirectly measured by the FSH concerns only the biologically active estrogens.

Further studies are necessary for the establishment of the estrogen level in women with uterine cancer and the influence it may possibly have in the development of the tumor.

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CLOSING REMARKS

JULES ANDRÉ BRET and FERNAND COUPEZ:

Munguia and Esther Franco, by administering the follicle stimulating hormone, arrived at the same conclusions as we did by giving the chemical hormones themselves. This raises the problem of estrogens of extra-ovarian origin, especially from the adrenal cortex.

Therefore, the study of all estrogenic and adrenal hormones will be most interesting in women who have undergone subtotal hysterectomy without conservation of the ovaries. This would permit one to compare the urinary output in those patients who develop a carcinoma of the cervical stump with those who have a normal cervix.

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH CERVICAL CARCINOMA

LUIS MONTALVO-RUIZ
Madrid, Spain

The cytologic smears from women with carcinoma of the uterine cervix contain not only cancer cells but also normal cells as well as cells with non-malignant alterations. The latter changes may appear in the cytoplasm, the nucleus, or may be apparent from the altered staining reaction of the cells.

In this presentation we will not concern ourselves with the malignant cells seen in smears of cancer of the cervix but only with the non-malignant cells present in such smears; namely, normal or completely benign cells and cells with non-malignant alterations in the cytologic specimens from premenopausal and postmenopausal women.

Material

The cytologic smears from 490 women with cancer of the cervix were studied in the University Clinic of Professor Botella during the period from 1949 to 1959.

Results

The normal cytology in invasive carcinoma and "in situ" carcinoma is somewhat different. In the latter case the cells are better preserved, since the smears are cleaner, with less necrosis and healthier flors.

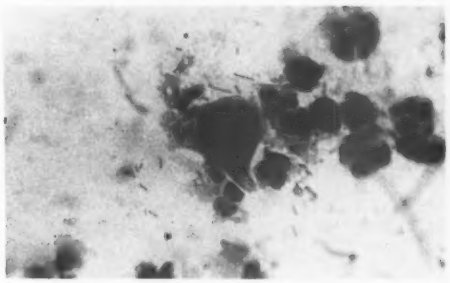

Fig. 1. Carcinoma of the uterine cervix. Atypical cell surrounded by leukocytes and Döderlein bacilli.

Even though some invasive carcinomas show healthy flora with many Doderlein bacilli, such as shown in Figure 1 in which we see Doderlein bacilli together with a cancer cell, we seldom make such observations.

In non-malignant cells with reversible alterations, we can see hyperchromatism, cytoplasmic vacuolization, changes in cell shapes, such as tadpole or fiber cells; and nuclear alterations, such as dyskaryosis, karyorrhexis, and degenerative pyknosis. All these changes can appear, besides carcinoma

		Average percentage according to type of cells found		
		Superficial	Intermediate	Parabasal
Premenopausal women	185	49%	38%	13%
Postmenopausal women	305	28%	33%	39%
	490			

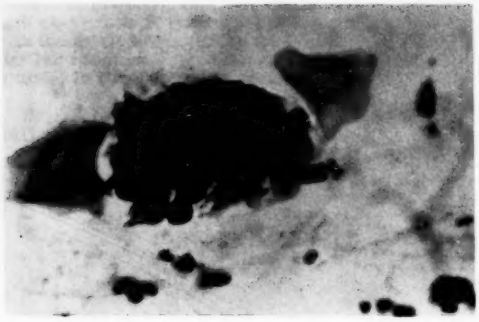

Fig. 2. Carcinoma in a woman of 32 years. Estrogenic smear type. Superficial cells and cluster of malignant cells.

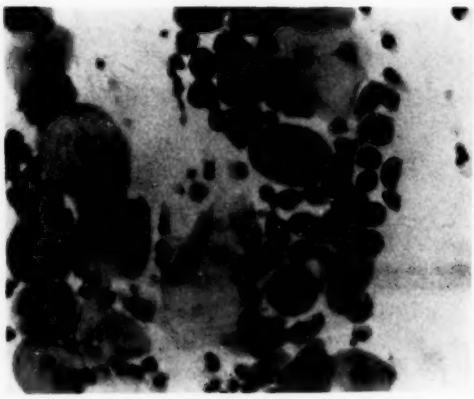

Fig. 3. Carcinoma-in-situ in a woman of 49 years. Atrophic smear type: Basal cells, parabasal cells, carcinoma cells and leukocytes.

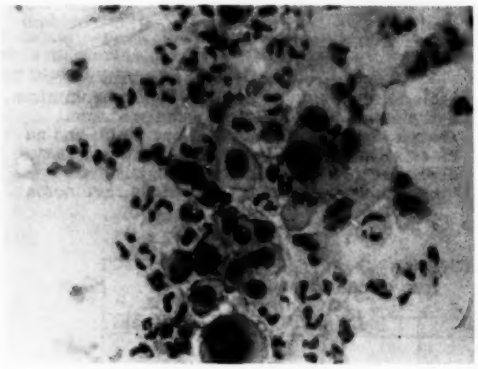

Fig. 4. Atypical cells in an atrophic vaginal smear.

of the cervix, in non-cancer cases such as dysplasias, inflammation, polyps, trichomoniasis and irritation due to pessaries.

Concerning the type of smear, we more frequently find the estrogenic type in premenopausal women (Fig. 2) and the atrophic type in postmenopausal women (Fig. 3).

We find these changes independent of age and type of carcinoma, and we interpret them as an inflammatory or proliferative or degenerative reaction concomitant with the carcinoma.

COMMENTARY: We find a slight elevation of the Karyopyknotic Index in women with carcinoma of the cervix. How can we interpret this phenomena?

Dingemanse et al. (5), demonstrated the presence of "Menoformone" on the skin of cancer patients since 1930, as well as in experimental tumors. With this basis some authors, such as Erica Wachtel (who also observed this elevation in the Karyopyknotic Index) think that cancer can produce estrogens which would be responsible for elevation of the Karyopyknotic Index.

Ruth Dearing (10) also points out the elevation of the Karyopyknotic Index in a group of women with carcinoma of the cervix in comparison with another similar group with other diseases.

Ayre (1, 2) and Papanicolaou (9) also find hyperestrogenic smears in corpus and cervical carconoma of the uterus.

In view of these facts, may we deduce that carcinoma increases the estrogenic level or do the estrogens induce the development of cancer? This is a problem which we cannot fully discuss in this short presentation. Numerous papers have been written regarding the role of estrogens in the etiology and the development of cancer, pointing out the coincidence of carcinoma of the uterus with endometrial hyperplasia (7, 8) with granuloma tumor (3, 6), and with estrogenic proliferation of the portio vaginalis (4). Up to the present time there is no definite agreement concerning this problem.

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HERBERT E. NIEBURGS
New York, New York, U.S.A.

During the era of exfoliative cytology many contributions have enriched this subject. I presume that the heading is directed towards the frequently reported presence of an increased number of karyopyknotic cells in cases of cervical carcinoma. This, in the manner in which it is widely reported, is of little significance.

In evaluating the importance of an altered percentage of normal cells in the vaginal smears of women with cervical carcinoma, those cases in which the identical cell types are normally found should be excluded as supporting evidence. During the proliferative phase, the finding of an increased number of karyopyknotic cells in the vaginal smear of a patient with cervical carcinoma is not significant. In addition, increased karyopyknosis in the presence of infection, particularly with coccoid bacteria, should be discounted as a significant correlative factor with cervical carcinoma. Some doubt is also cast on the validity of any relationship between karyopyknosis and cervical carcinoma in old age, since a high Karyopyknotic Index is frequently found in vaginal smears of many old women without any disease.

In a comparison of smears with increased karyopyknosis, of women with early or normal menopause between the ages of 41-60 and without carcinoma, to those with cervical carcinoma, only a slight increase in karyopyknosis could be found in those with cervical carcinoma. An appreciable increase, however, was noted in women past the age of 60. In the premenopausal age group, cell types, in both the vaginal and cervical smears of women with cervical carcinoma, were interpreted as showing persistent, slightly decreased estrogen activity, even when the vaginal smear contained an increased number of karyopyknotic cells. This interpretation was made in 71% of the women with cervical carcinoma in the premenopausal age group.

An interesting observation, which is perhaps more important than the factor of karyopyknosis, is the rare occurrence of vaginal smears with cytolysis in the presence of cervical carcinoma. In addition, it appears that vaginal smear containing lower intermediate and parabasal cells from women with cervical carcinoma is so rare that they, as well as the cytolytic smear, assume diagnostic significance, pointing to the absence of cervical carcinoma.

DISCUSSION

EMMERICH von HAAM, Columbus, Ohio, U.S.A.:

While the increased occurrence of endometrial carcinoma in menopausal women with relatively high estrogenic vaginal smears has been observed by us and many others, we have not observed a similar relationship in patients with carcinoma of the cervix.

HANNES KREMER, Vienna, Austria:

According to our experience, cells of all layers of the squamous epithelium were found in the smears of patients with carcinoma of the cervix. We could not find any significant increase of the eosinophilic superficial cells with pyknotic, centrally located nuclei, which demonstrates a sufficient estrogenic activity.

GILDARDO LEGORRETA, Mexico, D.F., Mexico:

I agree with Montalvo concerning the distribution of normal squamous cells in cervical carcinoma in premenopausal and postmenopausal women. Nieburg's observation on cytolysis and carcinoma of the cervix is quite interesting.

In cancer of the cervix the cells shed from the portion of the vaginal epithelium not affected by tumor permit a hormonal reading with the same limitations as in non-cancer patients. The limitations are increased by the presence of blood and necrotic material, when the carcinoma is invasive. From the point of view of the cytologic evaluation, there does not seem to be a definite hormonal pattern which permits suspicion of cancer of the cervix.

Concerning the higher Karyopyknotic Index mentioned in the main speaker's paper, I would like to ask if in such cases there is evidence of high estrogenic levels in the blood or the urine.

ALEXANDER MEISELS, Mexico, D.F., Mexico:

It is necessary to take into account not only the age group and the phase of the menstrual cycle, as has been pointed out, but also the type and grade of the cervical carcinoma, in order to evaluate properly the normal cell picture found in these cases.

It appears that in cases of in situ carcinoma of the cervix, the normal cells will show differential counts undistinguishable from those found in patients of the same age group without cervical lesion. Only rarely did the carcinoma in situ appear with slight elevation of the Karyopyknotic Index, in our series.

With invasive epidermoid carcinoma of the cervix, the differential counts are influenced by several non-hormonal factors:

1. Necrosis.
2. Infection
3. Erosion and ulceration in portions of the cervical epithelium that are free of cancer.

These factors depend most on the grade of the carcinoma.

In order to study the normal vaginal cells of patients with advanced or moderately advanced carcinoma of the cervix, it is advisable to take an extra smear of the vaginal wall of the lateral fornix, as compared with similar smears obtained from patients without cancer, in the same age groups, and/or in the same phase of the menstrual cycle. The preliminary impression, from a still reduced series of cases, is that there is a definite tendency towards higher karyopyknotic levels in the cases with carcinoma of the cervix. But this is not absolute, since a few cases were found with atrophic smears.

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HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D.F., Mexico:

We are going to mention only the cytohormonal pattern and the Karyopyknotic Index of the vaginal smears of patients with uterine cancer.

As other authors, we have found a slight increase of the K.I. in menopausal women with uterine cancer, as compared with normal menopausal patients. This observation had been reported previously by Papanicolaou (2), Ayre (3), and Dearing (4), and later confirmed by us (1).

In further studies, we have seen the importance of selecting the patients by the length of the menopause and not by age only, because of the variable cytohormonal patterns that can be found in menopausal patients. Our results are in Table I.

As the smears of the proliferated estrogenic type have a good percentage of karyopyknotic superficial cells (20 or more), the table indicates that in the women with 6 to 10 years of menopause, the percentage of smears with high Karyopyknotic Indices is greater than the indices observed in normal women at the same period.

If we consider that the smears of intermediate type are related to an epithelial proliferation produced by a slight but continuous estrogenic stimulation and not to an absence of hormonal effect, it can be deduced that a greater number of smears with estrogenic activity exists in women with uterine cancer (77.7%), as compared with normal women (60%).

If we consider the matter from this point of view, the reported data have a strong agreement with the results obtained with the chemical determinations of the urinary metabolites and the biological determinations reported by us in the discussion on the same topic.

The findings of the main speakers, confirmed by us, make one suppose that a greater estrogenic activity exists in the menopausal women with uterine cancer, as compared with normal controls. Nevertheless, as the number of cases studied is small, we believe that the problem is not solved yet and that further studies are necessary to make the

TABLE I

Women at the menopause with uterine cancer:

Length of menopause	Type of Smear			
	Atrophic	Intermediate	Proliferated	
1 to 5 years	4 (20%)	12 (60%)	4 (20%)	20
6 to 10 years	4 (13.3%)	18 (60%)	8 (26.6%)	30
11 to 20 years	6 (16.6%)	24 (66.6%)	6 (16.6%)	36
More than 21 years	14 (35%)	20 (50%)	6 (15%)	40
			Total -	126 cases

Women at menopause without uterine cancer:

Length of menopause	Type of Smear			
	Atrophic	Intermediate	Proliferated	
1 to 5 years	11 (25%)	24 (56%)	8 (19%)	43
6 to 10 years	12 (33%)	19 (53%)	5 (14%)	36
11 to 20 years	24 (53%)	14 (31%)	7 (15.5%)	45
More than 21 years	9 (56%)	5 (31%)	2 (12.5%)	16
			Total -	140 cases

statement that there is an abnormally greater estrogenic activity in women with uterine cancer, or that this influences the development of the tumor.

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CLOSING REMARKS

LUIS MONTALVO RUIZ:

All of the participants in the discussion agree with us except von Haam, who has not observed high Karyopyknotic Indices in cases with cervical carcinoma, but he has found estrogenic patterns in endometrial carcinoma. We cannot find any hormonal explanation for such differences in the vaginal pattern of both uterine carcinomas.

According to Munguia and Esther Franco the Karyopyknotic Index is slightly higher in menopausal women with carcinoma than in healthy menopausal women.

THE LEVEL OF ENDOGENOUS ESTROGENS IN PATIENTS WITH OVARIAN OR ENDOMETRIAL CARCINOMA AS DEMONSTRATED BY METHODS OTHER THAN EXFOLIATIVE CYTOLOGY

JULES ANDRÉ BRET
and
FERNAND COUPEZ
Paris, France

PETER STOLL
Heidelberg, Germany

For endometrial carcinomas we used the same methods of dosage as for cervical carcinoma, and we could make the same remarks about the date given by the chlorhydric and enzymatic hydrolysis.

Dealing here only with the general tendency of the phenol steroids to increase in the case of carcinoma, we found the following:

Women in reproductive age

level considered as normal: less than 90
total number of dosages: 17
dosages over 90: 15 (under 88%)

Menopausal women

level considered as normal: less than 10
total number of dosages: 50
dosages over 10: 46 (under 92%)

Therefore, in the majority of endometrial carcinomas the level of estradiol-estrone-estriol is much higher than normal.

The menopausal period is particularly demonstrative, since these high levels are not physiologically explainable.

Investigation in estrogenic stimulation by means of histology:

Carcinoma of the endometrium:	446 cases
Functional diagnosis possible:	62
Menopausal:	349
Climacteric:	55
Reproductive:	42
Without function:	39
Hyperplastic:	4
Proliferative:	13
Secretory:	6

Carcinoma of the ovary:

Estrogenic stimulus with hyperplasia of the endometrium is well known in Osteoblastoma (granulosa-cell tumor, theca-cell tumor). The endocrine function of the tumor does not indicate the grade of malignancy(2).

Granulosa-cell carcinoma:	17 cases
High malignancy (metastases):	5
Low malignancy:	12
Hyperlasia of the endometrium:	4
Hyperplasia of the endometrium:	11

Sertoli-cell tumor:

In 1956 we described two cases of malignant tumors of the ovary exhibiting androgenic and estrogenic efficiency as well (4.69 gamma/g and 3.09 gamma/g estrogen; and 1.6 gamma/g and 20.0 gamma/g neutral 17-ketosteroids) (1).

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COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH OVARIAN OR ENDOMETRIAL CARCINOMA

EKKEHARD KOFLER
Vienna, Austria

I. During reproductive age:

Endometrial carcinomas are not very frequently found in women in the reproductive age (2). In these cases the vaginal smear shows mostly a pattern compatible with the respective phase of the cycle. Thus, one finds in the first half of the cycle a more or less marked estrogenic activity, and during the second half of the cycle a picture compatible with progestational activity. Previous investigations (2, 5, 8, 9, 10, 14) show that carcinoma of the uterine corpus frequently arises in women with normal menstruational conditions. In these cases often young corpora lutea and an endometrium with high secretory activity have been found in addition to the carcinoma.

II. During the climacteric:

This episode in the life of the woman is characterized by frequently occurring anovulatory cycles, as a consequence of which the progesterone formation is missing while estrogens are produced. Approximately 20% of the carcinomas of the uterine corpus arise during this age and very frequently one will find a high estrogenic activity in the smears, just as one frequently finds, in addition to carcinoma of the endometrium, a glandular-cystic hyperplasia (4, 7, 11). We know, however, that during this episode, without an endometrial carcinoma being present, a high estrogenic activity and a glandular-cystic hyperplasia frequently may be encountered.

III. During menopause:

Since it is common experience that carcinoma of the uterine corpus occurs in the majority of cases during menopause and since, on the other hand, in postmenopausal smears estrogenic activity can most easily be recognized, we have concentrated our investigations on this type of material. A too high estrogenic activity has been reported (1, 6, 10) in more than one-half of the cases; however, this was in a small series of patients. On the other hand, other investigators (9, 13, 15) found an estrogenic smear pattern or a high estrogenic activity only in a much smaller percentage of postmenopausal patients with endometrial carcinoma.

In the following we give a preliminary report on our investigations still under way : Only those cases of endometrial carcinoma during menopause have been evaluated which are two or more years in the menopause. We have estimated the percentages of the various cell types but, on the other hand, in every case also counted 200 cells. We found that the estimated values did not differ from the counted ones.

TABLE I

Number of cases	Estrogenic Type	Mixed Type	Atrophic Type
102	5	16	81

Results of vaginal smears of women with endometrial carcinoma during menopause.

a. Estrogenic type: As a criterion for estrogenic stimulation we have evaluated only the eosinophilic karyopyknotic superficial cells (12).

b. Mixed type: Smears containing cyanophilic superficial cells and intermediate epithelial cells. Within this there are also included cells of the so-called "androgenic type" (10).

c. Atrophic type: These smears contain cells from the outer basal type and the basal type as well.

In three of the cases with "estrogenic smear type" a feminizing tumor was found in addition to the endometrial carcinoma, thus explaining in these three cases the abnormally high estrogenic activity.

The fact that the vaginal smear is atrophic in the majority of patients with endometrial carcinoma during the menopause is not surprising, since one also finds an atrophic and non-functioning endometrium in these cases (3, 8, 9).

Possible sources of error: When the smear is not taken from the posterior or lateral vaginal pool but from the vaginal wall close to the introitus, one often diagnoses an estrogenic smear type without an abnormally high estrogenic activity being present. Similar errors may arise in patients with prolapse or descensus.

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DISCUSSION

HÉCTOR MUNGÚIA and ESTHER FRANCO, Mexico, D.F., Mexico:

In twenty-three postmenopausal patients with endometrial carcinoma, we have found 37% of atrophic type of vaginal smears and 50% of intermediate type with results quite comparable to those of postmenopausal women without carcinoma.

Our results are most identical to those obtained by Wied (1).

The high percentage of vaginal smears of atrophic type found by Kofler in postmenopausal patients with endometrial carcinoma, shows more hypoestrogenism than those smears of postmenopausal women without cancer.

The results are interesting in view of the considerable number of patients studied by the author.

Together with Wied, we believe that a "standard of criterion and material" is necessary for evaluating the vaginal smears of these cases, in order to draw definite conclusions.

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VIOLETTE M. NUOVO, Paris, France:

Endometrial Carcinoma

1. During the reproductive age or the climacterum - There were 36 cases of endometrial carcinoma among the non-menopausal patients who had vaginal smears examined in our laboratory. We had roughly the same results as Kofler.

2. During the postmenopause - We found 131 cases of endometrial carcinoma among the postmenopausal patients who had vaginal smears examined in our laboratory. One hundred and two of these cases had a careful study of the smear hormonal pattern; there were patients who were postmenopausal at least two years and who did not have a hormonal treatment prior to the smears. The other 29 cases were not suitable for such a study: four cases had smears showing mostly blood and cancer cells, four cases were postmenopausal a rather short period of time, and 21 cases had had hormonal treatment. We compared the results to the results of a study made on 100 non-cancerous patients, who were postmenopausal at least two years and who had no gynecological infection or treatment. This group is purposely a different one from the one investigated in a previous paper.

Our above results are quite different from those obtained by Kofler.

	Total	Estrogenic type more than 30% pyknosis	Crowded menopausal type	Intermediate menopausal type	Atrophic menopausal type
Without carcinoma	100	2	43	8	42
With carcinoma	102	31	17	9	45

Definition of the above terminology:

"Crowded Menopausal Type": The general appearance resembles the premenstrual type. The cells are crowded and form dense groups. The nuclei are rather large and the Karyopyknotic Index generally does not exceed 10 to 15.

"Intermediate Menopausal Type": This group consists of cells of the intermediate type (described according to Papanicolaou), some of them are navicular cells. Few superficial and outer basal cells may be found.

Ovarian Carcinoma

There were 39 cases of ovarian carcinoma among the patients who had vaginal smears. Thirty-two of them were postmenopausal patients. The hormonal pattern has been evaluated in 19 cases:

Estrogenic type 5

Crowded menopausal type. 5

Intermediate menopausal type . . . 2

Atrophic menopausal type 7

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ANDRÉE PELZER, Liege, Belgium:

We are rather surprised by the high incidence of atrophic smears found by Kofler in his patients with endometrial carcinoma during menopause. We can only report the results of a series of 23 patients in conditions similar to his patients (at least two years postmenopausal); however, as small as such a series may be, its results show a different trend:

Number of superficial Karyopyknotic cells	Number of patients
0	1
1 - 10	15
11 - 20	4
21- 30	1
31 and more	2
	23

The smear without any superficial cells was of the mixed type.

Violette Nuovo reported a Karyopyknotic Index of over 30 in the smears of 33.6% of menopausal women with adenocarcinoma of the endometrium. In the great majority of our cases the Karyopyknotic Index was between 1 and 30, so that some disagreement between Kofler's and our results cannot be denied.

I wonder if the percentages of the various cell types can safely be "estimated."

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NO CLOSING REMARKS

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH UNTREATED, TREATED OR RECURRENT BREAST CARCINOMA

JOHN A. FINKBEINER
New York, New York, U.S.A.

In my experience, the cell type in vaginal smears of patients with breast cancer is not significantly different from patients without breast cancer or from other control groups, provided the groups are comparable in age and menstrual status. As Wied indicated in his discussion of Hormonal Evaluation of Patients With Endometrial Carcinoma (1), "It is not the purpose of this presentation to enter into the discussion of whether or not steroid hormones are associated with the occurrence of . . . breast cancer. This is especially not feasible since the cytohormonal observations on the vaginal epithelium . . . cannot identify the actual hormonal condition at the time of the onset of the lesion, but only at a period some time after its onset." Carrying this line of thought a bit further, one can say that the cytohormonal pattern of the vaginal cells is in no way related to the breast cancer but reflects the endocrine environment in which the cancer is growing.

The following groups of "age and endocrine-history-matched" women have been statistically evaluated:

1. Patients with primary operable breast cancer (prior to operation).
2. Patients with primary inoperable breast cancer.
3. Patients who had primary operable breast cancer treated by radical mastectomy and have had no evidence of recurrent disease.
4. Patients with recurrent operable breast cancer.
5. Patients with recurrent inoperable breast cancer.
6. Patients with benign lesions (usually chronic cystic mastitis).
7. Apparently normal women with breast complaints but no evidence of significant breast disease (seen in a cancer screening clinic).
8. Patients with a variety of other cancers of non-genital or non-endocrine organs.

None of the women had received additive or ablative endocrine therapy.

Vaginal smears in this and subsequent presentations were evaluated by the differential cell count technique, which records the percentage of cornified (eosinophilic, karyopyknotic superficial cells), pre-cornified (cyanophilic, karyopyknotic superficial cells), intermediate, parabasal (same as Graham's outer layer basal cell) (2), and basal cells (same as Graham's inner layer basal cell). While the division into five cell types is somewhat arbitrary, although one cell type merges imperceptibly into the next and the distinct limits of a given cell type may be difficult to fix, the differential cell count is a useful technique and allows one to document the entire morphologic pattern by careful enumeration of the cell types.

The concept of being able to express the entire cellular pattern of the smear has been an extremely useful one. To me, the Karyopyknotic Index or Eosinophilic Index limits one's expression, simply because they represent only a portion of the smear - not the entire pattern. This is particularly true when the evaluation of extremely low levels of endocrine activity become important.

To facilitate statistical evaluation, the Vaginal Smear Index was used. This code number is obtained by multiplying the recorded percentage of cornified cells by the factor 5, the percentage of precornified cells by a factor of 3, the percentage of intermediate cells by a

Vaginal Smear Index	Descriptive Category	Definition of Terms
> 3	Normal	Level of activity seen in normal ovulatory phase.
2.5 - 3.0	Low normal	Slightly less activity than normal, similar to that seen in mid-follicular phase.
2.0 - 2.5	Early menopausal	Level of activity seen pre- or post-menstrually or early in the menopause.
1.5 - 2.0	Menopausal	Level of activity seen in many women within 0-5 years after last menses.
1.0 - 2.5	Postmenopausal	Level of activity seen in many women 5-10 years after last menses.
0.5 - 1.0	Moderately atrophic	Level of activity seen in many women 10-20 years after last menses.
0.25 - 0.5	Markedly atrophic	Minimal levels of hormonal activity.
0.0 - 0.25	Completely atrophic	Absence of hormonal activity.

factor of 2, the percentage of parabasal cells by a factor of 1, and the percentage of basal cells by zero. These five products are totaled and divided by 100 yielding the Vaginal Smear Index. Although this Index is empirical, it is useful in that it provides a numerical expression of the total vaginal smear pattern. This pattern is expressed in terms of the following descriptive categories:

In each of the above groups of patients, about 60-70% of the women had vaginal smear patterns compatible with the age and menstrual history of the remaining patients, about half of the remainder (-15 - 20% of the total) had vaginal smear patterns showing much less activity than would be expected and the other half (15-20%) had higher levels of activity than seemed compatible with the stated endocrine history.

In summary, the vaginal smears of patients with breast cancer are not significantly different from the chosen control groups.

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DISCUSSION

PIERRE J. HAOUR, Lyon, Rhône, France:

Our experience with patients with breast cancer is limited to 60 cases from the Centre Anti-Cancereux of Lyon. These patients belong to the groups 1 to 5 evaluated by Finkbeiner.

For practical reasons, smears from the urinary sediment were considered, instead of vaginal smears. In our experience with urocytogram, the Karyopyknotic Index in the normal menstruating woman is not above 15.

	Atrophic	Karyopyknotic Index under 15	Karyopyknotic Index over 15	Totals
Between 50 and 60 years of age	6	9	8	23
Between 60 and 70	5	14	5	24
Over 70	1	5	7	13
Totals	12	28	20	60

This Table belongs to discussion of Dr. Haour.

We have been trying Finkbeiner's code for vaginal smears and we found it very simple and practical. Since urinary smears exhibit lower values for the Karyopyknotic Index than vaginal smears, we preferred in this report not to use it.

We found the following cytohormonal patterns:

Atrophic : 20%
Karyopyknotic Index under 15 (without basal cells) : 46%
Karyopyknotic Index over 15 : 33%

If the age of the patients is considered: (See preceding table)

In the group of 20 patients with Karyopyknotic Index over 15, 11 had Karyopyknotic Indices which were abnormally high: six of the patients are over 60 years of age.

It is difficult to compare our results with Finkbeiner's, but we can, however, consider the 12 patients over 60 years of age who had Karyopyknotic Indices over 15 as having higher levels of hormonal activity than seemed compatible with the dates of their menopause.

These 12 out of 60 patients represent the same percentage (20%) as the one given by Finkbeiner.

Comparing 148 smears of healthy postmenopausal women we found, in women between 60 and 70, 20% of cases with Karyopyknotic Indices over 15, and 10% of these cases in patients over 70 years of age.

As it was proposed by Wied (1) about the type of smears in endometrial carcinoma, I agree that it would be necessary that the authors evaluate their material with the same definite criteria, in order to draw a definite conclusion.

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1. Wied, G.L.: Acta Cytologica 2:629, 1958.

HÉCTOR MUNGUÍA and ESTHER FRANCO,
Mexico, D. F., Mexico:

Our results are similar to those obtained by Finkbeiner in the group of pre- and postmenopausal patients with breast carcinoma (treated, untreated and recurrent breast carcinoma) and also in patients free from cancer.

These results were obtained on a group of more than two hundred premenopausal patients with breast carcinoma and 400 normal women of similar age and menstrual history.

Persistent estrogenic activity was found in 57% of 70 postmenopausal patients

TABLE I

ESTROGENIC ACTIVITY TABLE

"0" COMPLETE ABSENCE OF ESTROGENIC ACTIVITY	EPITHELIAL ATROPHY	91 to 100% B	Vaginal Smear Index (Finkbeiner)
"I" HYPOESTROGENISM	A) MARKED	50-90% B, remaining I; 0 to 2% K	0.3 - 1.5
	B) MODERATE	20-49% B, remaining I; 3 to 5% K	1.59- 2.5
	C) SLIGHT	11-19% B, remaining I; 6 to 10% K	2.55 - 2.8
"II" ESTROGENIC ACTIVITY	A) SLIGHT	86-100% I, 11 to 14% K	3.0
	B) MODERATE	15-59% K, remaining I; 5 to 10% B	3.1 - 4.0
	C) GOOD	60-85% K, remaining I; 4% or less B	4.0 - 4.7
"III" HYPERESTROGENISM		86 to 100% K, remaining I	More than 4.7

B: Basal Cells
I: Intermediate cells
K: Karyopyknotic cells

Note: If a cytological count falls into two adjacent categories choose the less pronounced one.
If the count falls into two separate categories, choose the intermediate one.
(This Table belongs to the paper by Drs. Munguia and Franco.)

with breast carcinoma. There is no substantial difference between this percentage and that obtained by us on 140 postmenopausal women free of cancer (60%).

Vaginal smears were evaluated by the differential-count technique, recording only three types of cells: karyopyknotic, intermediate and basal cells (parabasal and basal cells). If we take into consideration the fact that the author multiplies karyopyknotic cells by five, intermediate cells (precornified and intermediate cells of Finkbeiner) by three, and basal cells by one, we obtained practically the same values.

As there is no characteristic vaginal smear type in postmenopausal patients, we believe that it is reasonable to report only the degree of estrogenic activity and not to draw a relationship with the time of the menopause.

Therefore, our discrepancy is only the "descriptive category" and "definition of terms" concerning postmenopausal levels.

Table I has been very useful to us in evaluating the changes observed in the cytological pattern of patients with breast carcinoma submitted to surgery.

CLOSING REMARKS

JOHN A. FINKBEINER:

It is gratifying that the observations of Haour and of Munguia and Esther Franco confirm my impression that (1) the vaginal smears of patients with breast cancer are not significantly different from age-matched controls, and (2) about 15-20% of the study group have vaginal smear patterns showing materially greater activity than expected from the endocrine history. About the same proportion of patients had a cyto-hormonal level much lower than exprected. It seems rather remarkable that the percentage breakdown in these studies agrees so closely. Actually I had anticipated there would be a divergence of opinion similar to that presented in the endometrial cancer cytohormonal studies (Acta Cytologic 2:626-635, 1958). Perhaps with a greater number of discussants, there would have been less agreement.

The necessity for standardization of terms and indices is apparent. Haour is entirely correct in emphasizing necessity of uniform definite criteria in the evaluation of material. Perhaps I have been somewhat obstinate in avoiding the use of the "Eosinophilic Index" or the "Karyopyknotic Index." It is apparent that from the differential cell count technique as outlined in the main paper, these indices can be easily determined. However, as I have indicated, I believe it is essential to express the entire vaginal smear pattern, not merely a portion of it. The Eosinophilic and Karyopyknotic Indices are expressions of maximal activity. By their use, the cytologist says, in essence, "This patient is having 10% maximal activity" (or 2% maximal activity, or 30% maximal activity, whatever the case may be).

In addition to the limitation of expression that these terms impose upon the cytologist, I am not sure that either the "Eosinophilic Index" or the "Karyopyknotic Index" is an accurate estimation of estrogenic activity, unless the cytologist is concerned with relatively normal levels of endocrine secretion. Certainly as progressively lower levels of activity are encountered (i.e., less differentiation of the vaginal smear pattern), neither index adequately expresses the varying degrees of activity that may be encountered.

Unfortunately, Munguia and Esther Franco have misinterpreted my Vaginal Smear Index as applied to their own differential cell count technique. The "precornified cells" (cyanophilic karyopyknotic superficial cells) in my classification should be included in their "karyopyknotic cells," not their "intermediate cells." This lowers all figures in the "Vaginal Smear Index" column of their Table I. As previously stated, the Vaginal Smear Index is useful in that it provides a numerical expression of the total vaginal smear pattern. Since the factors were chosen empirically, I have no objection to any index they wish to use. However, since the range of activity in their index is a bit different than mine, let theirs be called the "Munguia and Franco Index," for the numerical indices, although similar, are not comparable.

My "definition of terms" was not intended to be absolute, but rather an expression of an expected level of activity for comparison with the actual endocrine history. For instance a "menopausal level" may be seen in the early follicular phase of a normal menstrual cycle, in a patient twenty years postmenopausal, in a patient after oophorectomy or in a patient after adrenalectomy or hypophysectomy - all of which have different implications, although the vaginal pattern is similar - a proliferative pattern with little evidence of differentiation. An example of this type of smear is shown in Figure 1 in the following topic. If nothing more, these presentations indicate the necessity of further efforts toward standardization of terms and indices.

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH BREAST CARCINOMA AFTER OOPHORECTOMY

JOHN A. FINKBEINER
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Oophorectomy is a valuable therapeutic procedure for some patients with recurrent, metastatic, or primary inoperable breast cancer, as evidenced by measurable regression of disease with concomitant relief of symptoms and clinical improvement. Perhaps even more important, the response to oophorectomy indicates the hormonal sensitivity of the tumor. An objective response to oophorectomy demonstrates that the tumor is capable of responding to the hormonal imbalance produced by estrogen deprivation and implies that other methods of endocrine therapy (such as ablative methods of adrenalectomy or hypophysectomy or the additive measures of androgen or corticoid administration) may be successful after relapse following castration. On the contrary, if such a patient fails to achieve measurable tumor regression following oophorectomy, it is unlikely that these additional methods of endocrine alteration will produce objective improvement (1).

By definition, prophylactic oophorectomy is performed in a patient prior to the appearance of metastatic or recurrent cancer. Since there are no tumor deposits present which can be measured, it is difficult to evaluate the response to prophylactic oophorectomy. The determination of the patient's response to oophorectomy is deemed so important to the future management of the patient that prophylactic oophorectomy or the administration of other steroids post-operatively is not recommended. Either deprives the clinician of this essential information concerning the hormonal sensitivity of the tumor.

All patients in this presentation had recurrent, metastatic, or primary inoperable breast cancer and were premenopausal at the time of oophorectomy. All postmenopausal patients and those who had previous hysterectomy or pelvic irradiation have been excluded. The patients who received either sex hormones or corticoids following oophorectomy will be discussed separately. All patients had an adequate number of vaginal smears, both pre- and post-operatively; cases of vaginitis were eliminated. The vaginal smears were evaluated by the differential cell count technique outlined elsewhere in this symposium.

The study group consists of 94 patients of whom 40 women showed measurable tumor regression following oophorectomy. The remaining 54 patients failed to demonstrate tumor regression and are classified as failures, although some experienced subjective improvement. The age range at the time of oophorectomy of the remission group was 33 to 54 years, of the failure group, 32 to 52 years; the median age of both groups was 43 years and the mean age, 42 years.

The pre-operative vaginal smears are summarized in Table I. Nineteen patients had definitely low levels of activity similar to that shown in Figure 1, even at the height of their cycle. This is the smear type commonly seen in women shortly after menopause. However these patients were having regular menses and had no history or clinical evidence of endocrinopathy. It is striking that only one patient of this group achieved objective remission following oophorectomy. This patient had bilateral ovarian metastases completely replacing both ovaries; in other words, the patient had an "auto-castration." The remaining women, with unexpectedly low levels of activity on serial vaginal smears, did not show tumor regression after castration.

Therefore, serial pre-operative vaginal smears, which show materially less activity than would be expected from the menstrual history, may predict failure of oophorectomy with a reasonable degree of accuracy. However, surgical castration is a simple pro-

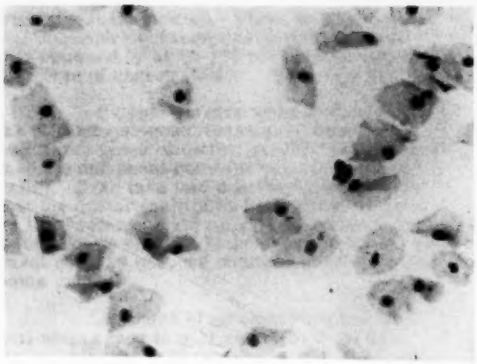

Fig. 1.

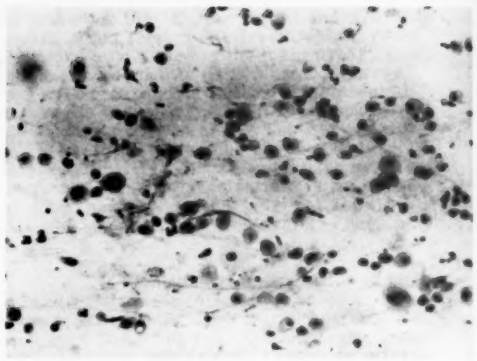

Fig. 2.

cedure with a low morbidity and low mortality rate and relatively free from the distressing side effects or complications of the other endocrine additive or ablative procedures. For this reason, the possible benefits of oophorectomy (including precise determination of the hormonal sensitivity of the tumor) has not been denied to any menstruating women with advanced breast cancer who is a reasonable operative risk.

Postoperatively the vaginal smears of different patients show a wide range of activity. The average vaginal smear pattern following oophorectomy is shown in Figure 1, a proliferative type of smear with little evidence of differentiation. This pattern is commonly seen in menopausal patients whose last menses occurred less than five years previously; the Vaginal Smear Index of this pattern is 1.5 - 2.0.

Figure 2 shows an example of the lower level of activity that may be seen following oophorectomy; this had been termed "moderately atrophic pattern" and indicates low levels of endocrine activity. The Vaginal Smear Index ranges from 0.5 to 1.0.

Figure 3 shows an example of a high level of activity following oophorectomy. From the degree of differentiation present, obviously considerable estrogen is being secreted. This is an example of "low normal"level of activity, with a Vaginal Smear Index of 2.5 - 3.0. This vaginal smear was obtained from an eight month post-operative patient. Although this patient failed to show measurable tumor regression following oophorectomy, some patients with only slightly less activity (Vaginal Smear Index 2.0 - 2.5) have achieved objective remission.

Within a few days of oophorectomy, vaginal smears begin to show progressively less activity. Occasionally an increased number of karyopyknotic cells may be seen. It is believed that this apparent increase in vaginal smear activity actually represents increased desquamation of the superficial cells secondary to hormone withdrawal.

About a month following oophorectomy, the progressive decrease in smear activity begins to level off. This may occur within two weeks after castration; occasionally it may last six months or longer.

Subsequent serial vaginal smears are constant in some patients, much less uniform in others. Assuming a relatively constant pattern in all patients, postoperative serial smears in 70 patients of this study group (28 remissions, 42 failures) approximated the "menopausal pattern." Four patients (2 remissions, 2 failures) had serial vaginal patterns higher than the menopausal level, and 20 patients (10 remissions, 10 failures) had patterns materially lower than the menopausal level. Therefore there is no significant difference in the postoperative vaginal smear patterns of the remission and the failure groups of patients. I have been unable to note any cytological criteria by which one could identify a vaginal smear, a patient who has achieved an objective response to oophorectomy from one who has failed.

Correlation of the vaginal smear pattern with the clinical course of the patient yields a great deal of useful information. If a patient has a vaginal smear pattern similar to that shown in Figure 3, and has steady progression in disease after oophorectomy, the tumor is growing in a rich estrogen environment. The patient may respond to other methods of endocrine therapy. On the contrary, good estrogen control has been achieved in the patient whose smear is shown in Figure 2. If such a patient has progression in disease, it is unlikely that further estrogen deprivation can be accomplished, and therefore such methods of treatment have little probability of success.

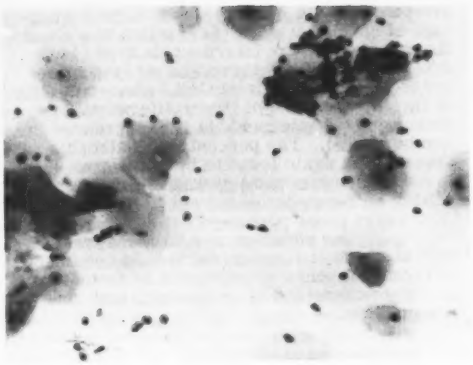

Fig. 3.

Of the forty patients in the study group who achieved an objective remission, thirty-five have subsequently relapsed, showing either progression in disease or new lesions. Eleven of these thirty-five patients showed an increase in vaginal smear activity preceding or simultaneous with relapse. In other words, serial vaginal smears may predict relapse in a patient who is enjoying a remission. All eleven patients showing this increased activity with relapse achieved a second remission when treated by other methods of estrogen deprivation.

It has been previously shown that remission rate in non-menstruating women is so low that oophorectomy per se is of little benefit (1). However some postmenopausal women will show unexpectedly high levels of vaginal smear activity. In these few selected patients, oophorectomy may produce an objective remission. All post-menopausal women who achieved a remission in the reported cases (1), had such a vaginal smear pattern. Additional cases have been noted. On the contrary, high levels of vaginal smear activity in a post-menopausal woman do not necessarily assure a remission following oophorectomy. This activity may be largely a reflection of extra-gonadal endocrine secretion and in this instance, would not be materially reduced by oophorectomy.

Some patients with advanced breast cancer will have had a previous hysterectomy for incidental reasons. Sometimes it is unknown if one or both ovaries have been removed. Serial vaginal smears may aid in the evaluation of this problem, although all the remarks and reservations in the paragraph above can be applied to this special situation.

Post-operative serial vaginal smears have been invaluable in detecting exogenous estrogen administration, perhaps unbeknown to the patient. Needless to say, the administration of estrogens nullifies the effect of oophorectomy. The vaginal smear response of an oophorectomized patient to estrogens is too well known to merit repetition and has been discussed in Acta Cytologica, Volume II, Number 2.

The administration of androgens or corticoids benefit some patients who have relapsed following oophorectomy. The effect of androgens on the vaginal cytology has been discussed in Acta Cytologica, Volume I, Number I. Neither oophorectomy nor breast cancer alters this response.

In a small group of women with advanced breast cancer in relapse following oophorectomy, six patients had an objective remission with corticoid administration and thirty-four had no measurable regression of disease. The corticoid administered was prednisone or prednisolone in maintenance doses of 20 mg per day, preceded by higher doses. In all six instances of remission, decrease in vaginal smear activity (compared to the pre-treatment level) was noted. Of the 34 failures to corticoid therapy, the vaginal smear activity remained essentially unchanged in 26 patients, was decreased compared to the pre-treatment level in six patients, but was higher than the pre-treatment level in two women. In both of these patients, there was an increase in differentiated cells, indicating increased estrogenic activity.

In summary, vaginal smears are a useful adjunct in the evaluation of a patient with advanced breast cancer undergoing oophorectomy, predicting failure in patients whose serial vaginal smears show an unusually low level of activity for their age and menstrual

TABLE I

PRE-OPERATIVE VAGINAL SMEARS

	Remission Group	Failure Group	Total Group
Normal range	35	27	62
Less than expected activity	4	9	13
Definite low	1	18	19

status. When correlated with the clinical findings, serial vaginal smears may predict relapse in certain patients enjoying a remission following oophorectomy and may indicate a method of therapy.

It should be emphasized that vaginal smears reflect the endocrine environment of the tumor. They do not specifically determine the susceptibility of a given mammary cancer to hormonal alteration but are useful in reducing the uncertainty of endocrine therapy.

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PIERRE HAOUR and
MARCEL DARGENT
Lyon, Rhône, France

It appears from the experience of many cytologists that oophorectomy in non-cancerous patients suppresses the action of estrogens in about one-third of the patients. The largest percentage of smears after castration is of the intermediate type, which does not exclude the action of estrogens on the vagina but which is also produced by other steroids, such as progesterone or testosterone.

A similar percentage of atrophic smears was found by us in women with cancer of the uterus after oophorectomy (elsewhere in this symposium).

In patients with carcinoma of the breast after menopause and without hormonal treatment (61 cases) we found only 14% with atrophic smears. There was a large percentage of normal smears (42%) which was about the same as for the intermediate type (44%). After oophorectomy there was an evident decrease in the Superficial Cell Index: only 23% of the smears were of the proliferative type; smears of the intermediate type increased in number (46%). The percentage of atrophic smears was again found to be of 30%, as seen in non-cancerous patients and in patients with cancer of the uterus.

Our experience with cases treated with steroids is limited, but we did not find a different response to estrogens or testosterone in oophorectomized cancerous and non-cancerous patients.

DISCUSSION

HÉCTOR MUNGUÍA and ESTHER FRANCO,
Mexico, D. F., Mexico:

Serial vaginal smears of 23 premenopausal patients suffering from breast carcinoma, submitted to oophorectomy, have been reviewed.

The results obtained are summarized in Table I.

Our results in premenopausal patients with normal estrogenic activity and slightly low estrogenic activity before oophorectomy are better than those obtained by the author.

The results obtained by us in the group of postmenopausal patients agrees with the results obtained by Treves and Finkbeiner (1). Those patients with estrogenic activity might achieve a remission with oophorectomy,

TABLE I

PREMENOPAUSAL GROUP			
Pre-operative vaginal smears	Regression Group	Failure Group	Total
Normal estrogenic activity level	12	1	13
Slightly low estrogenic activity level	3	3	6
Markedly low estrogenic activity level	0	4	4
POSTMENOPAUSAL GROUP			
Estrogenic activity	3	3	6
Moderate hypoestrogenism	1	3	4
Marked hypoestrogenism	0	3	3

but the presence of estrogenic activity will not assure a clinical remission.

In those cases improved by oophorectomy, in which later there was a relapse of the disease, adrenalectomy or administration of androgens might have produced a new remission.

There was decrease of estrogenic activity in most of the cases improved by oophorectomy; on the contrary, those cases not improved by the procedure did not show significant change of estrogenic activity.

In summary, it is our belief that serial vaginal smears are useful together with the clinical criteria in order to decide which patients might be improved by oophorectomy.

To decide if there is hormonal dependency, it is beneficial to perform oophorectomies in patients with advanced breast carcinoma, even in those cases with slightly low estrogenic level.

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CLOSING REMARKS

JOHN A. FINKBEINER:

Haour and Dargent and Munguia and Esther Franco have made interesting and pertinent observations that emphasize many of the difficulties that have been troublesome to me. The discussion in the previous topic, emphasized the necessity for standardization of terms and indices in the evaluation of vaginal smears. In this and the following two topics, the difficulties are compounded, for the vaginal smear data must be correlated with the clinical response. If cytologist seem to have somewhat different criteria for the evaluation of the vaginal smear response to hormonal alterations, clinicians seem to have even less agreement as to the definitions of remission and failure to a therapeutic procedure. For example, in the group of premenopausal patients reported by Munguia and Esther Franco, 65% achieved regression to oophorectomy, almost twice the percentage reported in the Memorial Center of Cancer and Allied Diseases of New York series. Since oophorectomy is a relatively simple surgical procedure, and since bilateral oophorectomy causes immediate and complete cessation of ovarian function, the only rational explanation for such a tremendous difference in the remission rate of premenopausal women must be a difference in criteria of evaluation.

As the previous topic indicated, Munguia, Haour and I use slightly different methods of vaginal smear evaluation. On this basic difference is now compounded an even greater variant - the clinical evaluation of the response of the patient. When one considers these difficulties, I am amazed at the basic similarity of the results. Unfortunately, the data I presented were restricted to premenopausal women. The response in non-menstruating women was discussed only in general terms. Therefore, complete comparison of these series is not possible. However, we are in accord that vaginal smears are a useful adjunct in the evaluation and care of a patient with advanced breast cancer, particularly those patients receiving endocrine therapy.

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH BREAST CARCINOMA AFTER ADRENALECTOMY WITH OR WITHOUT ADDITIONAL OOPHORECTOMY

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The vaginal smear response of patients with breast carcinoma treated by adrenalectomy is much more difficult to analyze than the response after oophorectomy. The incidence of accessory ovarian tissue apparently is extremely low. Treves has been unable to find a single authenticated instance (1). Therefore oophorectomy immediately, totally and effectively withdraws the gonadal secretion from circulation. This is not true after adrenalectomy. Not only is accessory adrenal tissue common (2), but replacement therapy with corticoids is mandatory. The terms "adrenalectomy," "bilateral adrenalectomy" or "total adrenalectomy" are misleading since it is uncertain whether all adrenal tissue has been removed surgically.

The clinical data on the patients in this presentation have been previously reported (3). It should be emphasized that this was an experimental series, largely unselected cases. As the study progressed, criteria for selection of patients were established that today permit greater selectivity.

Of the 155 patients reported in this series, 16 women were having normal menstrual periods. Seven (44%) of these patients achieved an objective remission to simultaneous oophorectomy - bilateral adrenalectomy. The remission rate and duration of remission of this group of patients was the same as that of oophorectomy (4). Although the group was small, there seemed to be no benefit from the combined procedure to patients who were still menstruating. It seemed apparent that the survival of some of these women would have been lengthened if the adrenalectomy was deferred until the patient relapsed after oophorectomy. Therefore, it has become the established policy of the Breast Service, Memorial Center, to reserve adrenalectomy for non-menstruating women; oophorectomy is the procedure of choice for patients with advanced breast cancer who are still menstruating (1).

We have not performed oophorectomy and unilateral adrenalectomy. This does not seem to be sound physiologically and conceivably can make evaluation of the patient more complex.

Ten of these sixteen premenopausal patients had an adequate number of vaginal smears to permit statistical analysis. The pre-operative vaginal smears were similar to the smears of menstruating women discussed elsewhere in this symposium. Serial post-operative smears had some fluctuation in most patients but approximated a "menopausal level" in 2 patients, a "post-menopausal level" in 5 patients, and a "moderately atrophic level" in 3 patients. (See elsewhere in this symposium under *).

Our experience with adrenalectomy preceding oophorectomy is limited to two cases, neither of which is included in the reported series (3). The first case, a woman with normal menses, was operated on seven years ago. She failed to achieve a remission due to adrenalectomy, and the steady progression of disease was not affected by oophorectomy four months later. Vaginal smears on this patient were unsatisfactory due to infection. The other patient, a 59 year old woman eight years postmenopausal, had bilateral adrenalectomy in June, 1958 at another hospital and had been maintained on cortisone 37.5 mg daily orally. When seen in January, 1959 she had generalized osseous, generalized soft tissue. and pulmonary metastases. A single

* topic: The cell type in vaginal smears in patients with treated, untreated or recurrent breast carcinoma

vaginal smear dated 1/9/59 was interpreted as "postmenopausal level" (index number 1.3). She was then admitted to the ward and the cortisone was discontinued. The patient had only minor symptoms and signs of adrenal insufficiency over a seven day period. This suggests that accessory adrenal tissue was present and functional. During this period of time, serial vaginal smears were in the "moderately atrophic level" with indices varying between 0.5 and 0.6. Biochemical analysis for urinary estrogens were reported to be 3 gamma/24 hours total estrogen (Brown method). Bio-assay was reported to be 0.32 gamma/24 hours (5). Oophorectomy was performed on 2/4/59 and revealed bilateral ovarian metastases. Postoperatively this patient has shown measurable regression of soft tissue metastases but no change in the pulmonary or osseous lesions. Serial vaginal smear indices have ranged around 1.0 since the operation. This patient has been maintained on 75 mg cortisone daily since the immediate post-operative period.

Although generalization cannot be made from one case, it seems that this is sufficient evidence to state that bilateral adrenalectomy without oophorectomy is not warranted.

Fifty-one patients of the study group had previous therapeutic oophorectomy, 28 of whom experienced an objective remission but had subsequently relapsed. Eight of these 28 patients had a remission after adrenalectomy. Vaginal smears are available on twenty patients, six of whom had remission. Five of these six patients had increased activity of the vaginal smears with relapse; all were of the "menopausal level" or higher prior to adrenalectomy. Of the twenty-three patients classified as oophorectomy failures, three had objective remission to adrenalectomy. The pre-adrenalectomy vaginal smears of these three patients were of the "early menopausal level" or higher. Post-operative vaginal smears of both the remission and failure groups (after adrenalectomy) were usually lower than the pre-operative levels, but there seemed to be no correlation between the clinical response and the degree of decrease in vaginal smear activity. No patient whose vaginal smear was "moderately atrophic level" or lower pre-operatively achieved a remission after adrenalectomy.

Forty-seven patients had experienced a natural menopause, 1 to 20 years prior to adrenalectomy. Adequate serial vaginal smears are available in 35 women of this group, of which 13 achieved remission after combined oophorectomy and adrenalectomy. Seven women had a vaginal smear pattern indicating greater activity than would be expected from their age and menstrual history; six of these achieved objective remission. Post-operative vaginal smears, although usually fluctuating to some degree, were definitely lower than the pre-operative level in all seven patients.

Six patients of the post-menopausal group had much less activity of the vaginal smear than expected, usually the "moderately atrophic level." All six patients failed to combined adrenalectomy-oophorectomy. Post-operative vaginal smears of this group of six patients were approximately the same as the pre-operative level in three women, somewhat lower in one, and slightly higher in the remaining two.

Twenty-two women of the post-menopausal group had pre-operative vaginal smear patterns compatible with their age and menstrual status; seven achieved objective remission to the combined procedure. Again the post-operative vaginal smears showed fluctuation in most of the patients. In three patients, the range of activity was slightly higher than the pre-operative level, approximately the same in six patients, and lower in the remaining 13 patients.

There was an insufficient number of vaginal smears from the remaining 27 patients in the study group who survived adrenalectomy to permit adequate evaluation.

With progression of disease or the appearance of new lesions following adrenalectomy, androgens or additional corticoids have been administered to some patients. Twenty patients received testosterone proprionate 100 mg intramuscularly three times weekly for a minimum period of six weeks. Three patients received objective regression of disease of short duration. The vaginal smear response to this androgen therapy seemed to be no different from the wide range of response previously described in ACTA CYTOLOGICA, Volume I, Number 1. It should be recalled that West et al. have reported the conversion of testosterone to estrogens in castrated, adrenalectomized women (6).

Twelve patients, who received additional corticoids after relapse from adrenalectomy, had no striking or consistent alteration of the vaginal smear pattern that could be attributed to the corticoid therapy. One patient had a transient objective remission to this treatment. Cortisone, 100 - 300 mg/d, or prednisone, 10 - 20 mg/d, in addition to the cortisone replacement therapy, were the corticoids utilized. My experience with this particular group of patients, who are usually in a pre-terminal condition, is far too small to permit any conclusions.

In summary, serial vaginal smears are a useful adjunct in the evaluation of a patient with advanced breast cancer, both before and after adrenalectomy. It is important to constantly remember that an "adrenalectomized" patient is not synonymous with total ablation of all adrenal tissue and that the cortisone administered to maintain life may have an effect on the vaginal smear. Again let me repeat that the vaginal smear merely reflects the endocrine environment of the tumor and has no direct relationship to the breast carcinoma. The vaginal smear pattern must be correlated with the clinical status of the patient to obtain useful information from the data.

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Removal of the ovaries does not imply suppression of the estrogenic stimulus, as has been demonstrated by vaginal smears and hormonal assays. After oophorectomy for breast cancer only about 30% of the patients show atrophic smears.

Adrenals are considered as the site of production of estrogens or steroids which can influence the vaginal mucosa (ACTA CYTOLOGICA, Vol. I) after oophorectomy. Complementary adrenalectomy is proposed in order to suppress this second site of estrogen production.

The effect of adrenalectomy on vaginal cytology has been studied by several cytologists but their experience, apart from Finkbeiner's, seems limited (1, 2, 3, 4, 5, 6). The percentage of patients with atrophic smears was not always found to be the same but all agreed that in an appreciable number of cases the influence of a hormonal stimulus remains.

We have studied 40 cases which were followed from one to 32 months after adrenalectomy. Urocytograms instead of vaginal smears have been used for practical reasons, using the conversion formula according to Lencioni (7).

We have found these figures for different types of smears:

Normal or hyperestrogenic	- 12.5%;
Intermediate	- 57.5%;
Atrophic	- 30.0%.

About one-third of the patients have suppression of the estrogenic stimulus (30% atrophic smears). The majority of smears shows a pattern of the intermediate type which is considered to be due to a limited effect of estrogens or to progesterone and androgen influence.

The smears with a Karyopyknotic Index over 30% are reduced in number.

Our experience with non-oophorectomized patients is limited to a few cases. All the cases reported here had had their ovaries removed.

Smears were also studied in a group of patients after adreno-splenic transplantation. After transplantation of the adrenals into the spleen, estrogens are supposed to be destroyed in the liver and corticoids to remain active. The technique used (8, 9, 10) gives to patients the possibility of getting along without any substitutive therapy. In the 13 cases studied, there were no normal smears found; eight were of the intermediate type and five of the atrophic type.

In conclusion, after adrenalectomy, with or without splenic transplantation, the proliferative estrogenic type group is appreciably reduced and the number of atrophic smears remains about 30%. The intermediate type group increases and one can only conjecture on the origin of the hormonal stimulus conditioning this type of smear: i.e., accessory adrenals, storage in tissues, action of cortisone treatment.

We did not find any difference between the response to hormonal treatment in adrenalectomized, postmenopausal patients and oophorectomized, postmenopausal, non-cancerous patients.

The use of vaginal smears (or the urocytogram) is practical in controlling the effects of surgery on the secretion of estrogens. Its value as a prognostic means has not been considered as effective by others (3). However, in our experience, we can say that there was a relationship between the evolution and the type of smear: changes in the smears to a proliferative type were in most cases followed by recurrence of the disease, but until now we have not been able to draw any definitive conclusions (10).

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DISCUSSION

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The results obtained in our patients submitted to adrenalectomy are as follows:

Four patients improved by oophorectomy, obtained a new regression with adrenalectomy. Failure of adrenalectomy was found in a patient who did not respond to previous oophorectomy. The vaginal smear of this case showed marked hypoestrogenism before the surgical procedures.

The vaginal smears of those cases improved by adrenalectomy showed estrogenic activity in two cases and slight hypoestrogenism in the remaining two. Lower levels of estrogenic activity were found in these cases after the procedure.

Regressions were obtained in two premenopausal women with good estrogenic activity, in whom one-stage oophorectomy-adrenalectomy was performed.

No conclusions can be drawn from this small group of patients. Nevertheless, our results are similar to those of Finkbeiner.

CLOSING REMARKS

JOHN A. FINKBEINER:

There seems to be general agreement on this question. The results of these three series indicate that estrogenic activity is usually reduced following adrenalectomy. Complete correlation of the clinical and vaginal smear response in these series cannot be made but several additional conclusions can be drawn:

1. Although bilateral adrenalectomy usually caused a marked reduction in vaginal smear activity, complete abolition of activity does not occur in the majority of patients.
2. Continued levels of activity (proliferation, not differentiation) do not exclude an objective remission.
3. Progression of disease may occur in patients with atrophic smears.
4. Despite the above exceptions, there is generally an inverse relationship between smear activity and clinical response, particularly in those patients with "estrogen-dependent" tumors.
5. Further studies are necessary.

THE CELL TYPE IN VAGINAL SMEARS OF PATIENTS WITH BREAST CARCINOMA AFTER HYPOPHYSECTOMY WITH OR WITHOUT ADDITIONAL ADRENALECTOMY AND/OR OOPHORECTOMY

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This paper reports the vaginal smear response of 55 women with advanced breast cancer treated by hypophysectomy.

Hypophysectomy has been reported to produce measurable regression of disease in women with recurrent, metastatic, or primary inoperable mammary cancer (1, 2a, 2b, 3). The vaginal smear response after hypophysectomy is quite similar to the changes seen following adrenalectomy.

Accessory pituitary tissue has been reported in 51 of 54 patients studied (6). It seems likely that complete ablation of the pituitary is seldom accomplished. Therefore it is impossible to study the effect of total ablation of the pituitary in humans. Post-operative endocrine therapy is mandatory following hypophysectomy(7). This presentation reports the vaginal smear response after hypophysectomy, with corticoid and thyroid replacement. The use of pitressin tannate intramuscularly or insufflation of pituitary powder has no apparent effect on the vaginal smear pattern. Analysis of the vaginal smear response to hypophysectomy is further complicated by the use of different criteria for clinical evaluation than have been used in the evaluation of oophorectomy and adrenalectomy series (1). In this presentation, only the vaginal smear changes after hypophysectomy will be discussed, omitting the correlation with the clinical response.

Vaginal smears were evaluated by the differential cell count technique as outlined under topic "The cell type in vaginal smears of patients with untreated, treated or recurrent breast carcinoma."

The physiologic effects of hypophysectomy have been described and are used as criteria of adequacy of the operation (8). Serial vaginal smears complement the various parameters of thyroid function, pituitary gonadotropin estimation, and other pituitary function tests. Two patients in this study group had hypophysectomy but post-operatively failed to show the expected alteration in any of the parameters of pituitary function. Serial vaginal smears remained in the "menopausal level" in both patients, one of whom had a therapeutic castration, the other had a spontaneous menopause five years previously. Re-exploration of both patients, 2 1/2 and 4 months later, failed to reveal residual pituitary tissue in the sella turcica (9). After the second operation, pituitary function tests, including serial vaginal smears, remained unchanged.

An additional fifty-three patients had serial vaginal smears sufficient to permit evaluation of the effect of hypophysectomy. Six patients were pre-menopausal at the time of hypophysectomy and all had a normal vaginal smear pattern. Post-operatively serial smears revealed a "post-menopausal level" in five women and a "menopausal level" in the other patient.

Thirteen patients had a previous therapeutic oophorectomy. One patient with an "early menopausal level" of activity pre-operatively had

no significant change in the vaginal smear pattern post-operatively. Six patients had a "moderately atrophic level" pre-operatively; the post-operative pattern was unchanged in three, somewhat greater activity in one, and slightly less activity in two women. Three women had a "menopausal" and three a "post-menopausal" level of activity pre-operatively. Grouping the post-operative response of these six patients, two had no significant change, three had less activity of their smear pattern and one greater activity.

Nine women had induced menopause, either for incidental reasons or a prophylactic castration. The pre-operative studies of five were in the "menopausal level," three had a "post-menopausal type," and one a "moderately atrophic level" of vaginal smear activity. Post-operatively six had no essential change compared to their pre-operative smears and three had decreased activity.

Twenty-three patients had incurred a spontaneous menopause. The pre-operative vaginal smears were in the "early menopausal level" of activity in five, "menopausal" in eight, "post-menopausal" in seven, and "moderately atrophic" in three women. Post-operatively the level of activity increased in six, decreased in twelve, and showed no essential change in five patients.

One patient had previous therapeutic x-ray castration. Her vaginal smear pattern was in the "early menopausal level" pre-operatively, decreasing to a "menopausal level" after hypophysectomy. A previous oophorectomy-adrenalectomy had been performed on one patient. Before hypophysectomy, serial smears were in the "post-menopausal" pattern with slight increase in activity post-operatively.

Fifteen patients received estrogen therapy after relapse following hypophysectomy, usually estradiol benzoate 5 mg intramuscularly daily for a minimum of three weeks. Only three of these patients showed the typical overwhelming cellular differentiation usually seen with this dosage. Six patients achieved a cellular pattern with indices in the 3.0 - 4.0 range. The vaginal smears of two patients showed only slight increase in activity with estrogen therapy, and the smears of the remaining four women showed no significant change from the pre-treatment pattern. This is quite a different response than that seen in post-menopausal women with intact ovaries or in patients who have had oophorectomy or adrenalectomy.

Ten patients received testosterone proprionate 50 to 100 mg three times weekly for a minimum period of 3 weeks as therapy for progressive disease following hypophysectomy. None showed significant alteration of vaginal activity or evidence of "androgen effect" as previously defined (cf. ACTA CYTOLOGICA, Volume I., Number 1).

Five patients received additional corticoid therapy above the maintenance therapy, cortisone 200 - 300 mg/d or prednisone 40 - 100 mg/d, with no significant change in the vaginal smear pattern.

It is interesting to note that this therapy with estrogens, androgens or corticoids failed to affect the course of the disease, causing neither remission or exacerbation in any of these patients.

Summary:

The pre- and post-operative vaginal smear response has been reported on 55 patients with advanced breast cancer treated by hypophysectomy. The group is too small to permit statistical analysis. Following relapse from hypophysectomy, treatment with estrogens, androgens and corticoids failed to effect the course of disease and often had little or no effect on the vaginal smear pattern.

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It has been reported that patients suffering from advanced breast cancer in whom remissions have been obtained with oophorectomy, may also respond with remission to hypophysectomy. It is also generally accepted that in those patients who did not respond to oophorectomy, no remissions have been obtained with hypophysectomy (1).

This report is concerned with the cytological pattern before and after hypophysectomy.

Four patients with advanced breast cancer were submitted to hypophysectomy at the General Hospital of Mexico City.

Vaginal smears were taken daily, before and after the surgical procedure.

Careful clinical evaluation was done in each case.

Hormonal determinations, I_{131} uptake, PBI, and ACTH test were done before and after

the procedure to judge if the hypophysectomy was complete from the functional standpoint. Cortisone-acetate was administered post-operatively (dosage: 25 to 50 mg/daily).

Of the four patients studied, three were oophorectomized previously without benefits. The fourth had spontaneous menopause of nine years duration.

The four patients had a cytological pattern of epithelial atrophy (80 to 100% basal cells).

The hypophysectomy was judged as complete in the first two cases. In these cases the cytological pattern remained unchanged as far as epithelial atrophy was concerned; nevertheless, an increase of size and more transparency and vacuolization of the basal cells were observed. Also, the vaginal smears appeared cleaner than before the surgical procedure.

The basal cells observed are quite similar to those described by Papanicolaou (2) as being of cervical origin, and remind one of those smears seen in post-menopausal patients receiving androgens.

The third case was considered as "incomplete hypophysectomy." At one time 75 mg of cortisone acetate were given, and simultaneously, an important change in the cytological pattern was observed. There was a decrease of basal and a predominance of intermediate cells (60 to 85%). No eosinophilic, karyopyknotic superficial cells were observed.

The morphology of the intermediate cells belongs to that observed in epithelial proliferation without complete maturation.

The fourth patient was also considered as "incomplete hypophysectomy." She had spontaneous menopause of nine years duration. Her cytological pattern varied considerably post-operatively. There was a decrease of basal with increase of intermediate cells. Eosinophilic, Karyopyknotic superficial cells appeared (about 10%).

The hypophysectomy did not improve the clinical course in any of our patients.

In summary the cytological findings are as follows:

A. Alteration of the morphology of the basal cells but no change in the picture of epithelial atrophy, in cases of "complete hypophysectomy."

B. Modification of the picture of epithelial atrophy and morphologic changes of the intermediate cells, in cases of "incomplete hypophysectomy."

No definite conclusions can be obtained from this work; nevertheless, some suggestions on the mechanism of the changes observed can be made:

We believe that the cytological changes observed in our patients are probably due to the cortisone administered, rather than the surgical procedure itself.

The resemblance of the morphology of the basal and intermediate cells after hypophysectomy, with those of patients with epithelial atrophy undergoing androgen therapy (3), suggests the possibility that some fraction of the administered cortisone could be transformed into androgens, or that there is an autonomic function of the adrenal cortex in respect to the production of androgens, or that there may be both mechanisms present. This theory is supported by the fact that there was diminution of the values of 17-ketosteroids, but never to zero, in two cases, and in the remaining two cases no changes were observed.

The absence of estrogenic hormones would favor the response of the vaginal epithelium to small quantities of androgens.

The appearance of eosinophilic, karyopyknotic superficial cells in one of our cases, suggests the possibility of transformation of cortisone to estrogens (4).

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NO CLOSING REMARKS

OVARIAN FUNCTION FOLLOWING PELVIC IRRADIATION AS ASSESSED BY VAGINAL SMEARS

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Our experience is based on two different groups of patients. First group: 35 female patients with cancer of the uterine cervix treated according to the Paris technique (Curie Radium Institute-Regaud technique), which comprises vaginal and intrauterine applications of radium (7200 to 8000 mg) on five consecutive days; and x-ray administration (12000 r) after the radium treatment.

In all of these patients as a part of the radiation response (RR) investigation program, vaginal smears were obtained weekly during the entire period of treatment (four to six months).

We have observed:

1. A steady and slow diminution of the trophism of the smears, manifested in a progressive lowering of the Eosinophilic and Karyopyknotic Indices. They behave during this time in a similar manner. At the end of treatment we can observe pictures of moderate to intense hypotrophia (Fig. 1).

MODIFICATIONS OF THE KARYOPYKNOTIC INDEX DURING TREATMENT

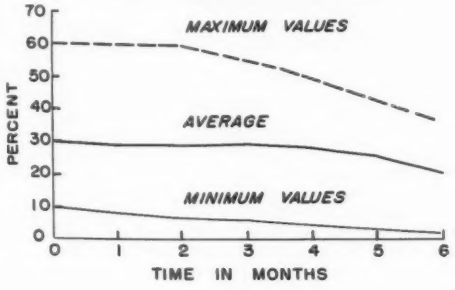

Fig. 1.

2. In six cases during the first month after radium application was removed, we have observed a transitory elevation of the Karyopyknotic Index for an average duration of 15 days. The Eosinophilic Index does not show a similar behavior in all of the six cases.

Second group: We have vaginal smears obtained from 87 female patients treated one to 15 years previously for cancer of the uterine cervix, by the same technique as described above. We have observed that:

1. The development of atrophic cytological pictures is slow and in our observations they are present only two or more years after treatment.

2. There is not seen as intense atrophic smears as we are accustomed to see after surgical castration.

3. The atrophic pictures (light to moderate in the sense of De Allende and Orias - 1947: 1 to 15% and 15 to 40% of parabasal cells present, respectively - are in direct relationship to the time elapsed since the treatment.

4. There is no relationship between the atrophic picture and the age of the patients at the time the smears were obtained.

Conclusions:

Apparently from these observations it is deduced that ovarian function is depressed and abolished by ionizing radiations but the effects are not as abrupt and impressive as after surgical castration.

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Estimation of residual ovarian function following pelvic irradiation is often difficult and inexact. With oophorectomy, gonadal secretions are immediately, totally and effectively withdrawn from the circulation and yet a tremendous variation in the follow-up vaginal smear pattern may be seen. This is shown in the topic in this Symposium entitled, "The Cell Type in Vaginal Smears of Patients with Breast Carcinoma After OOpherectomy." Functioning accessory ovarian tissue is extremely rare or non-existent. Therefore, following bilateral oophorectomy (after the post-operative period), any residual vaginal smear activity must originate from extra-gonadal sources.*

A similar wide range of response in vaginal smears occurs following pelvic irradiation. How much of any given amount of smear activity can be ascribed to residual ovarian function and how much should be attributed to extra-gonadal sources?

Unless serial vaginal smears show cyclical variation with characteristics of the menstrual cycle (possibly at a lower level of activity), there are no reliable cytological features to differentiate gonadal from extra-gonadal endocrine activity. The clinical history may give valuable information. Was the pelvic irradiation administered primarily to the ovaries or were the ovaries incidently irradiated in the treatment of some other lesion? If ovarian irradiation was intended, was the field accurately localized? What was the dosage and physical factors and characteristics of the irradiation administered? What was the estimated dose of irradiation delivered to the ovaries and in what period of time? Too often, the amenorrhea following pelvic irradiation has been interpreted as cessation of ovarian function. Return of menses and even pregnancy with normal progeny can occur following ovarian x-ray irradiation (1, 2). This is true particularly if tissue doses of 500 - 1000 r have been used. Even a tissue dose of 2000r (1000 kv, HVL 3.8mm Pb, delivered in 2 weeks) has failed to totally irradicate ovarian function as evidenced by an objective response to subsequent surgical castration in patients with recurrent inoperable breast cancer (1). Similar studies are in progress to determine if 3000r tissue dose (1000 kv, HVL 3.8mmPb, delivered in 2 weeks) will be effective (4).

Serial vaginal smear studies in these patients have yielded some helpful information. If the vagina is shielded to minimize the radiation effect on the vaginal mucous membrane, suppression of ovarian activity occurs about six weeks following the completion of the irradiation. These changes vary with the quality and quantity of radiation. Without this shielding, the radiation effect is so intensive that residual ovarian function cannot be assessed until the radiation effect has regressed. Following this, if the smear is entirely proliferative with practically no evidence of differentiation of the vaginal cells, it is likely that residual ovarian activity is minimal. Differentiation of the exfoliated cells indicates estrogenic activity. Assuming that this is entirely endogenous estrogen, it may be difficult or impossible to differentiate between residual ovarian function and adrenal activity.

* Since the completion of the discussion, my attention has been directed to a paper by Wharton (5), reporting two cases of supernumerary ovaries and one case of accessory ovaries as well as summarizing the world literature. The paper emphasized the rarity of this condition but certainly refutes the statements made that we were unable to find any proven instance of accessory ovarian tissue. An excellent bibliography of the reported cases in the English literature is furnished.

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After pelvis irradiation, either by radium or x-rays, the vaginal smears show a more or less marked regression of the hormonal function of the ovaries; but after some time, this first period of hormonal regression can be followed by a return of more or less important hormonal stimulation of the vagina. With the exception of cytological reaction of the RR-type, the different smear pictures, which can be observed after irradiation therapy, are identical to those observed during spontaneous menopause or after surgical castration. The vaginal reactions differ, however, in general, from those observed in the latter conditions, in that the estrogenic regression is more rapid than after spontaneous menopause, but less rapid than after surgical castration. Furthermore, the degree of persisting vaginal atrophy after massive irradiation is slightly more pronounced than after spontaneous menopause, but in general, complete vaginal atrophy, as observed rather frequently after surgical castration, is rare (1, 2, 7, 9, 10, 11, 12).

The present study is based upon the cytological control of 63 patients who received the usual castration dosage of x-rays or intra-uterine radium for functional bleeding, of 32 patients treated by radium and/or x-rays for uterine cancer without hysterectomy and of 24 patients treated by x-rays to complement a previous Wertheim operation. All patients have been followed by repeated vaginal smears for at least two years after the irradiation. This study permitted the following conclusions:

1. The degree of initial regression of estrogenic activity, as revealed by vaginal smears be-

fore and after treatment, is rather proportional to the applied dosage.

2. The persistence of the vaginal regression after treatment is not proportional to the applied irradiation dosage, but depends mostly upon the age of the patient or the previous activity of the ovary. It differs, therefore, from the vaginal regression observed after surgical castration. A massive irradiation by x-rays (a minimum of 3,000r for each ovary) associated with an intra-uterine application of 7,200 mgh radium can be followed by a return to a rather important hormonal stimulation of the vagina, if the patient is young and previously had normal ovaries. On the other hand an irradiation of 600r per ovary in a premenopausal patient with previous hypo-estrogenic vaginal smears can be sufficient to produce a persistent vaginal atrophy similar to that observed after surgical castration of patients of the same age.

3. In patients submitted to irradiation treatment by intra-uterine radium application at a castration dosage (2,000-3,000 mgh), for functional bleeding, the induced amenorrhea is more the result of a direct destruction of the endometrium by the radium than of destruction of the hormonal activity of the ovaries. Indeed, the vaginal smears in such patients only show in rare cases (in older patients over 50 years of age) a corresponding persistent vaginal atrophy, while younger patients frequently show a rapid return to a more or less normal vaginal smear. This conclusion, that artificial menopause by radium treatment and even by x-rays at so-called castration dosage is, in general, an organic amenorrhea caused by destruction of the endometrium, has been demonstrated by application of the same radium dosage in the vagina at the same distance from the ovaries (8). In six patients treated by purely intravaginal application of 3,000 mgh radium, this treatment had no effect upon the abnormal bleeding or the endometrium, except in one case (48 year old patient), while in six other similar patients intra-uterine application of the same dosage of radium produced a persistent amenorrhea in all six cases. The variations of the vaginal smears were identical in all 11 patients (with the exception of the 48 year old patient), so that one could conclude that the vaginal epithelium is much less sensitive to irradiation than the endometrium, that the irradiation did not change the previous ovarian function, and that the amenorrhea resulted only from a direct action of the radium upon the endometrium and not from a castration of the ovaries. This has been proved by test administration of estrogens in the "castrated" patients where the endometrium no longer responded to estrogens, as shown by biopsy, while the vaginal cytology responded in a normal manner to the injected estrogens.

4. In young patients treated by massive irradiation of the ovaries (x-rays or radium at complete cancer dosage), this treatment is usually followed by a persistent amenorrhea. In these cases, therefore, one could conclude, a priori, that the treatment has resulted in a complete block of the ovarian hormonal activity. But even in these patients a persistent vaginal atrophy is rare, at least rarer than after surgical castration. In many cases, the vaginal smears show again, after a transitory period of atrophy, pictures corresponding to a rather important genital hormonal activity. In some patients the smears again become of the typical estrogenic type, in others they show typical androgenic reactions, and in others they present pictures similar to that of normal pregnancy, with the difference being that the cells have a marked transparency of the cytoplasm, the nuclei are more hypochromatic and the cell clusters are not as thick as in the typical pregnancy. These smear pictures can sometimes present difficulties for a differential diagnosis between pregnancy and climacteric amenorrhea (13). I have called them "pseudo-pregnancy smears" (10). As the same smear types have been observed in patients after hysterectomy alone or after Wertheim operation followed by massive x-ray treatment, the hormonal origin of these smear types cannot be the ovaries, but seems to be the adrenal cortex (4, 7, 9). The latter conclusion has been proved by hormonal assays, which in such patients, after Wertheim operation and massive x-ray treatment, have shown a rise of the urinary 17-ketosteroids to over 10 mg/24h, estrone eliminations up to 36 gamma/24h and in some cases even small quantities of chemically identified pregnandiol.

As the absence of ovaries in these patients can be excluded rather positively by the Wertheim operation, the origin of this new endocrinological activity could only be the adrenal cortex, and it seems that irradiation could stimulate it, as comparative studies show a higher frequency of androgenic and mixed androgenic smear types than after surgical castration.

5. In all 119 patients, the RR cells have been observed in the first weeks after treatment. the follow-up of these patients has shown that the degree of the RR reaction depends mostly upon the applied dosage, while the persistence of the RR cells is essentially related to the hormonal state of the vaginal epithelium before and after treatment. In patients with consecutive persistent vaginal atrophy, therefore, in general, in patients over 50 years old, RR cells can be found for as long as two years and even longer after irradiatiation, while in young patients with subsequent return to some hormonal stimulation of the vaginal epithelium, the RR cells disappear sometimes in less than three months, even if the patient had received a total dosage of 12,000r by x-ray and 7,200 mgh of radium. This relation of the RR reactions to the hormonal activity of the patient is similar to the partial relationship of the RR cells to the hormonal state of the patient (3, 5, 6). I think that this relationship of the RR and SR reactions to the hormonal state of the patient should not be forgotten in the prognostic evaluation during irradiation treatment of cervical cancer.

CONCLUSIONS

Irradiation treatment of the pelvis by x-rays or radium does not produce a complete vaginal atrophy or a complete disappearance of genital hormonal production. The resulting amenorrhea has mostly an organic, traumatic origin

by direct destruction of the endometrium. Irradiation treatment in younger patients is frequently followed, after a transitory vaginal regression, by a return of some hormonal stimulation of the vagina. As these vaginal reactions are observed, even in patients irradiated after Wertheim operation, the origin of this new hormonal stimulation seems to be the adrenal cortex. The degree of the RR reactions of the vaginal epithelium depends upon the applied dosage of x-rays or radium, but the persistence of the RR reactions is related to the hormonal state of the patient after irradiation treatment.

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DISCUSSION

WARREN R. LANG, Philadelphia, Pennsylvania, U.S.A.:

Therapeutically administered pelvic irradiation affects the vaginal smear mainly by its depressant effect on ovarian function. Other changes result from its direct action on the vaginal epithelium and on the epithelium of the cervix and of the endometrium. As is usual in other states, local infections may confuse the interpretation of the smear.

Both Finkbeiner and Arrighi emphasize that there is a wide range of response found in the vaginal smear post-irradiation and that the changes are not as abrupt as those following bilateral oophorectomy. As Pundel states, the persistence of smear changes following irradiation is seemingly correlated with the age of the patient. Intra-uterine radium application for benign bleeding in young women may permit the ovaries, which are probably only slightly affected, and the endometrium to recover sufficiently to allow subsequent normal pregnancy (1).

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ALEXANDER MEISELS, Mexico, D.F., Mexico:

It is clear from the previous papers that pelvic irradiation, even in doses used for therapy of cervical carcinoma, does not necessarily produce vaginal smears of the atrophic type. On the contrary, after an initial hypotrophic picture, a number of cases will show a marked karyopyknosis. The reappearance of an estrogenic effect is considerably more important in irradiated patients than in oophorectomy cases. This would seem to suggest that after irradiation the ovaries may resume a certain degree of activity. It is also possible, as suggested by Pundel, that the vaginal smear in these cases reflects a vicarious adrenal activity.

It is permissible to think that when ovarian suppression is not instantaneous as in surgery, the adrenal glands may take over part of the function of the ovaries. This would explain the higher estrogen activity found after irradiation, as compared to surgery. It might be the physiologic response of the adrenals to failure of the gonads during menopause. As this failure is gradual, it resembles more the suppression of the ovaries by irradiation than the sudden removal of the gonads by surgery.

One fact that has caught our attention in our series is that frequently a high Karyopyknotic Index in a patient irradiated for cervical carcinoma is associated with recurrence of the carcinoma. This finding has not been statistically analyzed yet, but it has happened with some regularity in our series and might have a prognostic significance. In some instances, a patient may have an atrophic or hypotrophic smear for many months, then superficial cells reappear, the Karyopyknotic Index rises sharply, and shortly afterwards malignant cells are found in the smear. But this sequence seems to be the exception: Once an atrophic smear has been obtained for several months, a recurrence is rare. The cancers that recur seem to have persistently high Karyopyknotic Indices that show little variation after irradiation. These findings confirm the results described by Erica Wachtel (1), who postulates the theory that the tumor itself is responsible for the estrogen production. An alternate possibility would be that higher estrogen levels provide favorable conditions for the growth of the tumor.

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GIUSEPPE MOGGIAN and UGO CITTI, Bologna, Italy:*

The study of ovarian function following pelvic irradiation has aroused a great deal of interest in recent years, thanks to new and improved methods of cytology. Thus it is that we have been able to do this study on a large amount of material, supplied to us by the Departments of Radiology and Obstetrics and Gynecology, University of Bologna, Italy.

* From the Department of Obstetrics & Gynecology of the University of Bologna, Italy.

We have observed a total of 60 women who received pelvic irradiation for genital neoplasms and hemorrhagic metropathia. Thirty-six received this for cancer of the cervix, 10 for cancer of the endometrium, and 14 for hemorrhagic metropathia.

Of the 36 patients treated for carcinoma of the cervix, we have been able to follow up 20 of them for some months after treatment, while the other 16 patients re-entered due to a relapse from one to four years after the initial treatment.

The technique used in the cases of cervical carcinoma consisted of irradiation of four fields, two-by-two opposite each other or with moving therapy on two fields, one anterior and one posterior, for a total focal dose of 3,000 - 3,500 r, in addition to an intracervical and intra-uterine application of 55-60 mgh of radium.

In the cases followed during and after treatment, we have been able to observe a regression of estrogenic stimulation within two months after the beginning of therapy. The persistence of the degree of regression appeared to be proportional to the state of ovarian function preceding the treatment and to the age of the patient.

In fact, in pre-menopausal or menopausal patients, there was never any help toward a resumption of ovarian function, while in the other patients, assistance was given to a greater trophism of the cellular elements which were mainly of the intermediate strata, with a sufficient number of elements of the lower superficial strata, almost all being cyanophilic. In some microscopic fields there were observed some rare eosinophilic superficial cells with pyknotic nuclei.

In the 16 patients that returned for observation after the first treatment, a variable cytological picture was seen. In the younger women, more time had elapsed and the estrogenic stimulation as revealed by vaginal smears, was greater. Not only this, but the regression appeared earlier and the cytological estrogenic aspect was proportionally greater. In patients undergoing menopause the usual senile phenomena encountered at this age were not observed, but the elements that were present were of an intermediate or parabasal type, the eosinophilic superficial elements being absent.

In 10 patients treated for carcinoma of the endometrium, the technique of which did not differ from that used for carcinoma of the cervix, the results of the vaginal smears after treatment demonstrated a more or less notable regression within approximately two months; and after four months, using serial smears, we did not observe any significant resumption of ovarian activity inasmuch as we dealt with pre-menopausal patients.

In the 14 cases treated for hemorrhagic metropathia, six were treated with 11 intra-uterine application of 50-55 mgh of radium. Amenorrhea was established from the first days of the treatment, without, however, the smears revealing any regressive transformations proportional to the amenorrhea.

The successive smears, on the contrary, denoted a progressive decrease of estrogenic activity, that at a certain point reached a more or less constant level; never, however, approaching that of physiological menopause, nor as rapid as in surgical menopause.

In the eight cases treated with definite x-ray castration, who had received a dose of 1600 r to each ovary, divided into four opposite fields (one anterior iliac and one posterior gluteal), the regression of ovarian function was proportional to the degree existing before therapy. In fact, in three cases, even after treatment, there appeared a more or less typical menstrual cycle, even though the estrogenic level was low; and only after three months had elapsed did the cytological picture become hypotrophic.

These were the three young patients who had a notable degree of preceding hyperestrinism. In the other six patients, however, the cytological regression was more marked and earlier, but these women were nearing menopause. Observations one year after definite x-ray castration revealed cytologically a discrete increase of estrogen activity in the cases of the three young women, and also in one case of the six pre-menopausal patients.

In one of the three cases there appeared a metrorrhagia one and one-half years after the x-ray castration, and endometrial biopsy showed a glandular hyperplasia. The treatment in this case was an intra-uterine application of radium.

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JOSÉ R. del SOL and CARLOS ROHRBACH, Madrid, Spain:

During the past year, the Cytology Laboratory of the Provincial Institute of Obstetrics and Gynecology of Madrid has studied smears of 156 patients who had undergone irradiation in the Radiotherapic Department, for malignant cases of the uterus. Of the total number, 42 cases, in which hormonal readings were not made for several reasons, have been set apart. In addition 14 cases had been given 10 mg of estradiol bezoate in order to facilitate the evaluation of cellular irregularities (proliferation test). Our finding relative to the hormonal situation of patients who had undergone irradiation refer to the remaining 100 cases.

For the hormonal evaluation we have followed the criterion of Wied. We have divided the patients into four groups, with the following cases corresponding to each one:

1. Evident estrogenic effect - 8 cases - 8%
2. Slight estrogenic effect - 18 cases - 18%

3. Strong hormonal deficiency - 26 cases - 26% (Similar to the atrophic menopausal type)

4. Intermediate Smear - 48 cases - 48% (Similar to the non-atrophic menopausal type)

There are no statistical differences as far as the age of the patient or the time since the irradiation are concerned. (It is to be noted that the time since irradiation was never less than one month nor more than six.)

As far as the type of radiation received and its possible relation to the hormonal situation are concerned, our findings are as follows:

	Radium only (12 cases)	Radiotherapy (49 cases)	Radium + Radiotherapy (36 cases)	Cobalt Bomb (3 cases)
M.E.E.	1	5	1	1
S. E.E.	2	11	4	1
A.M.T.	2	16	8	-
NA.M.T.	7	17	23	1

Key: M.E.E. - Marked Estrogen effect (1)
S.E.E. - Slight estrogen effect (2)
A.M.T. - Atrophic menopausal type (3)
NA.M.T. - Non-atrophic menopausal type (4)

In twelve our of the fourteen patients who had received 10 mg of estradiol benzoate days before the irradiation, the vaginal epithelium was highly proliferated. In the remaining two cases, the smear was similar to that of the non-atrophic menopausal type.

CLOSING REMARKS

JOHN A. FINKBEINER:

The interest shown by the main speakers and discussors reveal that the estimation of residual ovarian function after pelvic irradiation must be a rather common problem and there is a striking similarity in the submitted answers. I have failed to clearly state that all patients reported in my series had breast cancer; there were no patients with uterine cancer or functional bleeding.

It should be emphasized that vaginal smears do not differentiate ovarian from extra-gonadal endocrine activity unless:

1. The patient has had a bilateral oophorectomy - in which case any residual activity must come from extra-gonadal sources.
2. Serial vaginal smears show cyclical variation characteristic of the menstrual cycle.

In all other instances, I know of no criteria to differentiate residual ovarian function from adrenal activity.

Many thanks to the contributors for their interesting discussions.

J. PAUL PUNDEL:

There seems to be general agreement among the discussants that:

1. Pelvic irradiation for malignancy even by x-ray and radium treatments will not always be followed by complete estrogen deficiency. The degree of ovarian regression is an intermediate stage between that of spontaneous menopause and that of surgical castration.

2. Pelvic irradiation by x-ray alone has a more marked effect upon the hormonal activity of the ovaries than intra-vaginal or intra-uterine application of radium. The amenorrhea resulting from radium treatment is in most cases not the result of an ovarian regression, but of the direct local effect of radium upon the endometrium. Radium only has a very low penetrative effect and the distance separating the radium from the ovaries is usually too large to seriously affect the ovaries under normal conditions.

There remains the problem of the association of a high Karyopyknotic Index with a recurrence of carcinoma after irradiation treatment, as presented by Erica Wachtel and Meisels. This would be an interesting topic for discussion in a future symposium.

VAGINAL SMEARS IN THE MENOPAUSE

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The term menopause may be applied to (1) the last menstrual period, (2) the transition period of ovarian decline during which time menstrual periods cease ("change of life"), or (3) to the more or less characteristic but inconstant symptom complex of hot flashes, nervousness, headache and the like. It is probably more desirable to use the term menopause to indicate the change-over period, and the menopausal syndrome to designate the symptom complex that may occur. In the discussion of the "menopausal smear" that follows, the cytological changes described will include those found with the onset of the climacteric through the late postmenopausal era. As at other periods of life, the cytological smear mainly reflects the hormonal status of the individual although such factors as the aging process, nutritional status, and local processes such as infections and neoplasia play a role in modifying the smear.

The smear previous to the last menstrual period

For a variable time before the last menstrual period, as ovarian function diminishes the smear may appear perfectly normal and even demonstrate a sufficiently high Karyopyknotic Index with subsequent regressive changes to signify the occurrence of ovulation, even though the latter may be defective. Later, progesterone production fails and a hyperestrogenic smear type ensues. This will demonstrate many eosinophilic, karyopyknotic cells which may undergo cytolysis if Döderlein's bacilli are in sufficient abundance. It is at this time too that resistant mycotic infections may supervene.

Periods of climacteric amenorrhea may occur in which the vaginal smear may show squamous cells of the intermediate layers. These cells may crowd and fold and may therefore resemble a pregnancy smear. Wied has suggested the administration of estrogen to differentiate the two conditions since the amenorrhea of the climacteric, unlike that of pregnancy, will

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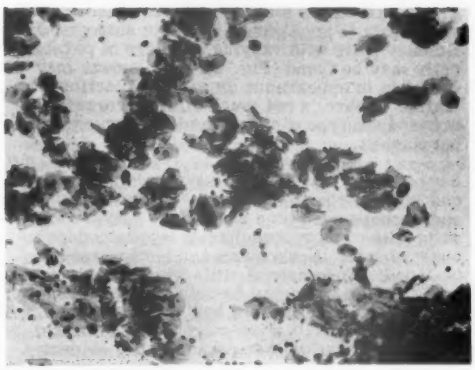

Fig. 1. Vaginal smear of a 56 year old woman who had a normal menopause ten years previously. Normal pelvic findings. Note the "regressive" changes with folding and crowding of intermediate cells. (x 120)

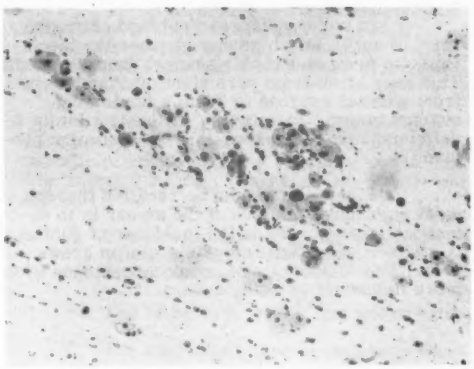

Fig. 2. Vaginal smear of a 72 year old woman who had a normal menopause twenty-four years previously. Normal pelvic findings. Note numerous parabasal cells with leukocytes. (x 120)

respond to hormonal stimulation. At times, but not usually, a truly atrophic smear with parabasal cells may be found, yet, some time later, the women have an episode of ovulatory or anovulatory menstruation.

The smear following the last menstrual period

In general, when the ovary is no longer able to produce sufficient estrogen, menstruation and the postmenopausal era ensues. The same can occur also should castration be performed either surgically or by irradiation. With surgery, the postmenopausal changes are more abrupt; with irradiation the smear may be appreciably modified by the effect of the irradiation itself.

There is no pathognomic cytological picture of the postmenopausal era, although as the woman grows older an atrophic smear is more likely to be found. For approximately a decade, however, the smear may indicate good estrogenic effect, with or without cytolysis. Regressive changes similar to those mentioned previously (Fig. 1) or an atrophic estrogen deficiency state with varying numbers of parabasal cells may be found (Fig. 2). The smear may fluctuate in appearance as ovarian function fluctuates. There is not much clinical correlation between the type of smear and the presence of hot flashes.

The atrophic smear when well pronounced is quite characteristic. Many parabasal cells and perhaps even true basal cells are present with cells of more superficial layers conspicuously absent. Leukocytes and histiocytes may be found. Endometrial cells which are normally scant at this time may be obtained by endometrial aspiration and may show hypochromatic nuclei. Endocervical cells demonstrate a similar change. With inflammation, when a clinically evident atrophic vaginitis is present, pseudocornification appears and leukocytes increase in number. At this time also the deeper cells may show more or less uniform nuclear enlargement and hyperchromasia; cytoplasmic vacuolization is not uncommon. Döderlein bacilli may be present even in the very old. Trichomonal and even Candidal infections can occur, although they favor women of the childbearing age group.

In some instances the blood estrogen level is apparently high enough after the menopause to prevent atrophic changes from occurring. This may arise from persistent ovarian function, from adrenal sources or from a feminizing ovarian tumor. In animals, at least, vitamin A deficiency may be responsible for a similar picture (2).

Finally, it should be recalled that the most significant mission of the smear is to detect malignancy. Since the incidence of genital tract carcinoma increases as a woman grows older, this aspect of smear taking becomes even more important as aging occurs.

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In investigations recently performed by us on vaginal smears of symptomatic postmenopausal women of 50 to 97 years of age, with a minimum duration from the cessation of menstruation of at least one year, the smears have been classified according to the scheme of Schmitt, which uses three degrees of proliferation.

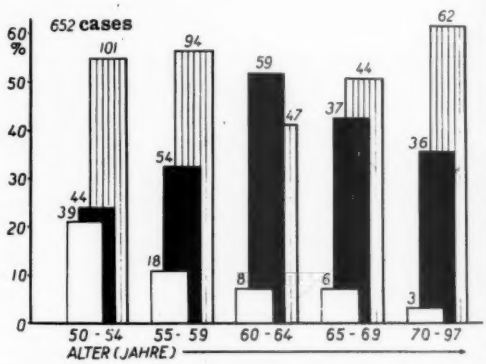

Fig. 1. Age and degree of proliferation of the vaginal epithelium. Light columns: Relative frequency of Group A (Schmitt). Hatched columns: Relative frequency of Group B (Schmitt). Black columns: Relative frequency of Group C (Schmitt). The number underneath indicates the absolute number of cases.

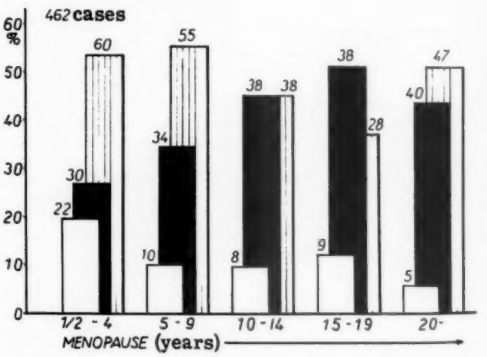

Fig. 2. Duration of the menopause and the degree of proliferation of the vaginal epithelium. Symbols as in Figure 1.

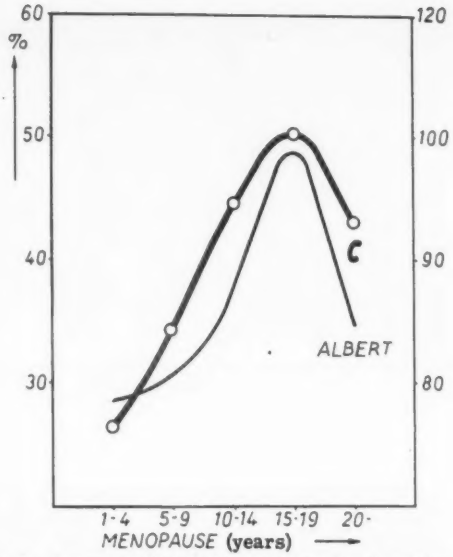

Fig. 3. Frequency distribution of Group C and gonadotropin excretion in the urine of postmenopausal women (after Albert). The thick line indicates the relative frequency of atrophic smear patterns; the fine line indicates the gonadotropin excretion for 24 hours in rat units under consideration of the duration of the menopause.

Group A - Schmitt 2-3, 3, 3-4
Medium proliferation

Group B - Schmitt 1-2, 2, 2-3
Minimum proliferation

Group C - Schmitt 1, 1-2
Atrophy

It becomes evident from the relationship between the frequency distribution of the particular cytological groups and the age of the women studied (Fig. 1) that the values of Group A decrease continuously with increasing age without, however, disappearing completely. The distribution of Group C and in the opposite manner the values of Group B reach their maximum between 60 and 64 years of age. An identical behavior can be shown also when the results are compared with the duration of the menopause (Fig. 2). The general validity of this observation is apparently affirmed by the following remarkable fact: The lack of estrogen production with atrophic smear patterns leads one to assume that there is a high secretory activity of the anterior lobe of the pituitary. Now, if one compares the curve of anestric types with the gonadotropin excretion in the urine of postmenopausal women (2), one immediately remarks at their surprisingly similar behavior.

The very coarse characteristic of these degrees of proliferation (Schmitt Classification) does not permit a decision as to whether or not the proliferation phenomena frequently occurring even in the senium, especially in Group B, are caused by estrogens or by androgenic substances. Their results, however, prove that between the ages of 60 and 65 the distribution curve, increasing until this point, again decreases and at the same time the relative frequency of the lower degrees of proliferation (Group B) again increases. Therefore, it seems very likely that this critical shift, which is also mentioned by Randall (3), may be due to a change in the secretory activity of the pituitary or to a compensatory activation of the adrenal cortex which is accentuated during this period.

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LUIS MONTALVO-RUIZ
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In several hundred menopausal women studied cytologically, the most varied smears were observed from advanced atrophy to the hyperfollicular smear. In 1956 (1), at the fifth meeting of the Spanish Gynecological Society, colpocytological findings in the post menopause were discussed, and we obtained thirty-three percent of the women with a smear of evident estrogenic activity. Our actual study comprises women more than a year postmenopausal, one being 83 years of age and 32 years postmenopausal. The age group ranges between 41 and 83 years of age. In this study we can group the results into four fundamental categories, in so far as frequency observed and its hormonal significance is concerned.

Group I: Smear of the Follicular type
Group II: Smear of the Intermediate type
Group III: Smear of the Parabasal type
Group IV: Smear of the Atrophic type

Group I: Figure 1. Characterized by an elevated proportion of superficial cells, sometimes as high as 70%, the rest formed by intermediate cells and one or more parabasal cells.

Group II: Cyanophilic intermediate cells predominate, with a scant proportion of superficial cells and some parabasal cells. In this category we can distinguish three other categories:

A. The intermediate with the above stated characteristics, represented in Figure 2.

B. The luteal type with piling and crowding.

C. The androgenic type of clear intermediate cells with hyperchromasia of the cytoplasm and nucleus, some of them crowded. Despite the fact that we call this an androgenic smear, the final affirmation will not be made until 17-ketosteroids are titrated. The infre-

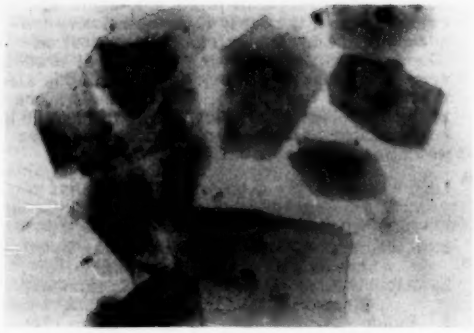

Fig. 1. Follicular smear type. Woman of 65 years of age. Nine years postmenopausal. Karyopyknotic Index 62.

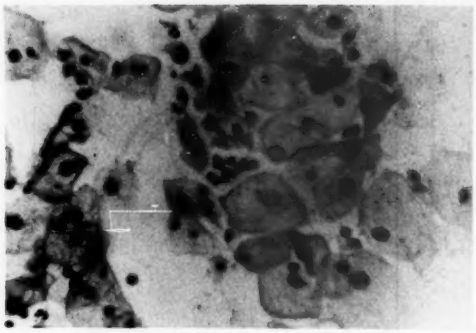

Fig. 2. Intermediate smear type. Woman of 52 years of age. Six years postmenopausal.

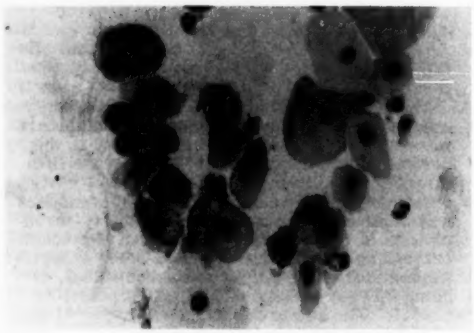

Fig. 3. Parabasal smear type. Woman of 59 years of age. Ten years postmenopausal.

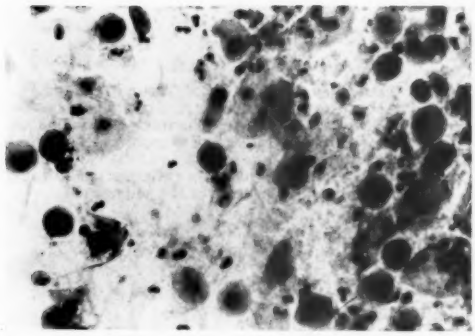

Fig. 4. Atrophic smear type. Woman of 58 years of age. Ten years postmenopausal.

quency with which we observe these smears does not justify a separate category.

Group III: Figure 3. Parabasal type. In this smear the cell predominance is based almost exclusively on an increase of parabasal cells with a scant proportion of intermediate and no superficial cells.

Group IV: Figure 4. Atrophic type. The smear is made up of deep cells without intermediate or superficial cells. These smears usually have a great amount of leukocytes and bacteria.

Comments: We know that this classification is much too sketchy, but with it we are trying to simplify the very many and complicated classifications now existing. Undoubtedly, in our classification not all the types of smears that we have been observing in the menopause are included, such as mixed smears that, by their precise variety, cannot be correctly classified. We observed, with some frequency, smears with cytolysis that, because they lack specific meaning, could not be used to establish a hormonal diagnosis: we do not form a separate category for these smears.

HANNAH PETERS
Copenhagen, Denmark

Investigations of smears taken on women during menopause show that a smear which can be called characteristic for menopause does not seem to exist. The impression that smears become atrophic after the cessation of menses and after estrogenic stimulation of the vaginal mucosa stops is not borne out by surveying

TABLE I

Classification of 122 Vaginal Smears Related to Age				
Age	Atrophic: No Estrogen Stimulation	Intermediate:	Mature:	Ungraded:
35-45	1	6	1	
46-55	17	28	9	1
56 and over	33	13	6	2
unknown	3	1	1	
Total	54	48	17	3

TABLE II

Classification of Smears Related to the Duration of Menopause				
Duration of menopause	Atrophic	Intermediate	Mature	Ungraded
6 mos.-5 yrs.	5 (23%)	14	4	1
6 yrs.-10 yrs.	6	14	4	
11 yrs.-20 yrs.	27 (55%)	15	7	1
21 yrs. and over	11 (69%)	4	1	1
unknown	5	1	1	

smears of menopausal women. Smears taken during menopause (when menopause is defined as the spontaneous and final cessation of menses that has lasted longer than six months in women over 35 years of age) vary greatly, and the type of smear to be found cannot be predicted either by the age nor by the duration of the menopause.

We screened vaginal smears taken on 122 women past menopause (1). These investigations were carried out on Indian women living in Bombay. The smears were classified into atrophic, intermediate and mature smears (2). The occurrence of either intermediate or mature smears was taken as evidence of persistent estrogen stimulation.

Of the 122 smears surveyed, 54 were atrophic, whereas 65 (53%) showed evidence of estrogenic stimulation (Table I). With advancing age an increase in the number of atrophic smears occurred in our series: 29% of the smears taken on women in menopause up to 55 years of age were atrophic. In women older than 55 years estrogen stimulation of the vaginal epithelium had ceased in 63% of the cases; 37% of the smears from women in this age group, however, still showed evidence of estrogen activity.

Correlating the smear type with the duration of menopause (Table II), it is noted that estrogen stimulation continues in the majority of women in the first ten years after stoppage of the cyclic bleeding; only 23% of the smears were found to be atrophic. Even in women who had been amenorrheic 11 years or more, a considerable number of smears showed estrogen stimulation: 41% of the smears were found to be either intermediate or mature in this group.

This indication of a continued estrogenic stimulation in Indian women after menopause, as revealed in the vaginal smear, correlates well with findings of Randall (3) in American women and Struthers (4) in English women. Randall reports evidence of estrogenic stimulation after menopause in 55% and Struthers in 58% of postmenopausal women, which compares well with the 53% found in our own survey. All three series indicate that a considerable number of women continue to show cytologic evidence for estrogen stimulation during menopause. It is, therefore, understandable that during menopause all types of smears characteristic for different levels of estrogen stimulation are found and that a smear type characteristic for menopause cannot be described.

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PETER STOLL
Heidelberg, Germany

In the following data we record the functional cytology results from 1111 patients in the menopause with the following requirements:

1. They had been in the menopause at least two years.
2. X-ray or radium had not been used before treatment.
3. By taking a precise case history, hormone-therapy within six months period previous to the vaginal smear could be excluded.
4. Clinical examination gave no evidence for the existence of an ovarian tumor.

The use of cytological methods on patients who are in the spontaneous menopause gives us information of a hormonal stage in which endometrium no longer responds to the ovarian hormones (bleeding is no longer produced). The vaginal epithelium is a precise indicator of the presence of sex hormones and cyclic changes can be analyzed even after the menstrual bleeding ceases. Occasionally these changes continue into the late menopause. On the other hand, one frequently finds a high degree of maturation of the smear preparation, and it can be assumed that the production of estrogen is not terminated when uterine bleeding stops. Histological evaluation has shown the same results. We have found, in postmortem studies of the uterus endometrium, of 196 patients over 65 years of age that 36 times a slight proliferation of the endometrium was present, while in 10 cases the endometrium was very proliferative. In all those patients the last menstrual period was finished more than 10 years before. The particular problem of such prostrated estrogenic stimulation lies in the observation that female proliferative hormones can stimulate the growth of tumors, particularly the development of uterine corpus carcinoma. If this assumption is correct, the finding of an estrogenic smear in the menopause could be an important indicator for the examiner. Erica Wachtel described a cornification index with high maturation of the cells in coincidence with genital carcinoma or recurrence of genital carcinoma. The subsequent hypothesis assumes that malignant tissue is capable of producing estrogen-like substances. In our opinion these findings may also result in disturbance of liver function in those patients with carcinoma, because it is well known that by a decreased decomposition of estrogens in the liver, the remaining small amounts of estrogen substances will produce cornification of the vaginal epithelium. In our patients, therefore, those with liver disturbances were also excluded. Further testing should be carried out - especially with reference to the results shown by Erica Wachtel in order to determine if the findings of increased estrogenic activity in tumors could be better explained by the fact that the decomposition-products of the tumor tend to flood the liver in such a manner that the estrogenic breakdown is no longer able to follow its normal course.

Our findings are divided according to the length of the menopause (2-10 years, over 10 years of age) and also according to a) patients with no sign of any kind of proliferative process within the genital area and, b) patients with presence of proliferative processes either benign (i.e., benign polyp) or malignant (i.e., carcinoma of different localization).

Influence of the length of menopause:

Duration	Estrogenic	Androgenic	Atrophic	Total
2 - 10 years	124 (21%)	328 (55%)	147 (24%)	599
10 - n years	70 (14%)	254 (49%)	188 (37%)	512
Total	194	582	335	1111

Androgenic means here middle-type proliferation with prevalence of intermediate cells.

Further subdivision into patients with proliferative and non-proliferative processes reveals:

Duration of Menopause-		Non-proliferative	Proliferative	Total
2 - 10	estrogen	46 15%	78 27.8%	124
	androgen	185 60%	143 49.2%	328
	atrophic	77 25%	70 23.0%	147
Total		308	291	599
11 - n	estrogen	32 10.8%	38 17.5%	70
	androgen	155 52.6%	99 45.6%	254
	atrophic	108 36.6%	80 36.8%	188
Total		295	217	512

In the almost equal percentage of atrophic smears in each group according to the length of menopause, the number of estrogen-stimulated in the proliferative processes is significantly increased as compared to the non-proliferative (healthy) cases (27.8% compared to 15% and 17.5% compared to 10.8% respectively). The continued existence of estrogen stimulation appears to be frequently connected with a proliferative process rather than with a lack of hormonal stimulation. This is especially true for the first year of menopause.

If one further divides the proliferative group in order to indicate the benign and malignant changes, the following becomes apparent:

Duration of Menopause		Benign	Malignant collum, vagina	Malignant Corpus uteri
2 - 10	estrogen	45 28.3%	18 25.3%	15 24.2%
	androgen	80 50.7%	32 45.0%	31 50.0%
	atrophic	33 21.0%	21 29.6%	16 25.8%
Total		58	71	62
11 - n	estrogen	15 16.8%	14 20.0%	9 15.5%
	androgen	49 55.1%	30 42.9%	20 34.5%
	atrophic	25 28.1%	26 37.1%	29 50.0%
Total		89	70	58

This tabulation indicates that a nearly complete agreement exists between the smears in the benign proliferation and those in the malignant proliferation. With reference to the length of menopause the decrease of the estrogenic effect in the proliferation processes is more accentuated than in the non-proliferation cases. From this, one might be able to conclude that a protracted estrogenic effect shortens the latent period in the onset of tumor-growth by its proliferative effect, if a tumor condition is present.

CONCLUSIONS

1. It is obvious that the atrophic menopausal smear in the menopause does not, by any means, correspond to the norm, but instead, numerous patients show a hormonally stimulated vaginal epithelium up to an advanced age. In the early menopause (up to 10 years) estrogenic stimulation is found in about 1/5 of all cases, in the late menopause in about 1/6 of all cases. The typical atrophic smear is found in 25% in the early menopause and up to 50% in the late menopause.

2. From the practical standpoint the finding of an advanced proliferative smear does not positively indicate the presence of a proliferative process, particularly not of a carcinoma of the genital tract. We found atrophic smears in 50% of our patients with adenocarcinoma of the corpus uteri (late menopause) and in 25.8% in the early menopause.

3. If a proliferative process is present, either benign or malignant in nature, estrogen stimulation of the vaginal epithelium is significantly more frequent than in healthy patients. This applies particularly to the early menopause, but also to the late menopause.

4. In the presence of a proliferative process the atrophic menopausal smear occurs with the same frequency as the estrogenic smear type during the first 10 years of menopause; however, the atrophic type is considerably more frequent in the late menopause.

5. Malignant growth has its own rules in-

dependent of the presence of proliferative hormones. Manifestation of the tumor, on the other hand, may be influenced by proliferative hormones, which might be able to shorten the latent period.

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DISCUSSION

ZELMA A. KALNINS, Winston-Salem, North Carolina, U.S.A.:

In full agreement with Lang, Luksch, Hannah Peters, Montalvo Ruiz and Stoll are our own observations that there is no pathognomonic cytologic picture for menopause, but that the completely atrophic smears, consisting only of parabasal cells, will most likely be found in women older than 55 years, who are more than 10 years postmenopausal.

I am not certain that the differences in smear patterns depend only upon different hormonal stimulations. Excluding pathological conditions, I presume that physiologically irritating factors, such as sexual intercourse, may cause proliferation of vaginal and cervical epithelium.

I wonder if there are statistically evaluated data available comparing cytological pictures of sexually active and inactive menopausal women.

Secondly, I am interested to know the opinions concerning the peculiar structures occasionally seen in atrophic smears of menopausal women. These structures are round or oval, 10 to 30 micra in diameter, staining deeply blue, the smaller ones resembling naked, hyperchromatic nuclei, and the larger ones showing a granular appearance and centrally located small, round, poorly distinguishable nuclei.

WOLFGANG KORTE, Bonn, Germany:

Menopause means that menstruations cease, due to a physiological depression of ovarian function. This is a process of involution. The onset and the duration of this involution vary among individuals very much, even if one compares women with pathologically unchanged genitalia. Also, the onset of menarche can vary from 10 to 18 years of age. This means that certain individuals need a longer period of development before their ovaries begin to function. Empirically, women with late menarche have an early menopause. This may be connected with a certain "stepmother-like" treatment of their genital organs, and may also be an expression of a general tendency of the organism toward early decomposition and aging. Then there are also women who have their first menstrual bleeding very early. Generally, in addition they maintain their ovarian function for a longer time. If we consider these two groups of women, then it is not justified to compare such individuals with the collective term "menopause."

By this I mean that the function of the ovaries is quite different in differently constituted organisms. Even if the function of comparable ovaries would be qualitatively and quantitatively the same (no one is able to demonstrate this), different results would occur due to the varying reaction of the end organs.

Because of these and other considerations, one should discontinue the statement that pictures of the vaginal epithelium in the menopause at a suitable degree of maturation are caused by estrogenic stimulation. It would be better to say "comparable to an estrogen effect." In addition, there are other factors of a local nature which make the smear directly or indirectly comparable to this effect. Thus, I would like to say that the normal individual disposition of a person to grow, to age, to degenerate, to regenerate, in short his biological disposition, determines to a great extent, in addition to the local factors, the variability of cytological vaginal pictures during menopause.

Nevertheless, I do not want to say, as does Hannah Peters, that "there probably does not exist a smear which could be considered as characteristic for the menopause."

Certainly the definitely atrophic menopausal type of epithelium is also cytologically characteristic for the pattern of a complete and perhaps rapidly occurring involution. This might not be so for all women in the menopause regardless of their age and their initial biological condition. But this smear pattern is present only in the physiological menopause, i.e., after involution of the ovaries. For this interpretation one needs no statement of age. The young, oophorectomized woman does not show this smear, because her general cellular and tissue consti-

tution, independent of the estrogenic ovarian stimulation, does not yet tend toward involution or atrophy. Only the smears of young female children show remotely comparable results. Also, in this case the experienced individual recognizes the strongly marked growth potential of the nuclei.

ALEXANDER MEISELS, Mexico, D.F., Mexico:

All five authors agree that a certain amount of estrogen activity is frequently demonstrated in vaginal smears of menopausal patients. The time elapsed since cessation of the menses seems to be important. Shortly after the last menstruation, hyperestrogenic smears are not rare. I agree with Lang that this is probably due to failure of progesterone production, which is also responsible for the suspension of menstruation.

When ten years or more have elapsed since cessation of the menses, and the vaginal smears still reveal estrogen activity, the question arises whether this estrogen is produced in the gonads or has its origin in extra-gonadal sources. Since cytologic evidence of estrogen activity is also found in women after irradiation of the ovaries, and in some cases even after oophorectomy, the second possibility seems to have more strength. That the adrenal glands could be the source of this estrogen has been indicated by surgical experiments in cases of cancer of the breast. The estrogen that can be demonstrated after oophorectomy in these cases disappears when both adrenals are also removed. It would appear that the adrenals take over part of the function of the ovaries when these gradually fail after menopause.

In spite of these facts, the question remains whether the gonads themselves are not, at least in part, responsible for the estrogen activity in late postmenopause, in normal cases.

Stoll's findings of higher estrogen activity in patients with proliferative processes is very significative. It points out once more that high estrogen activity in elderly women who are not receiving any hormonal treatment should be considered as an alarming sign that warrants a thorough search for a possible malignancy.

HÉCTOR MUNGUÍA and ESTHER FRANCO, Mexico, D.F., Mexico:

We agree with the main speakers that there is no one type of smear characteristic of the menopausal period. We have reviewed the vaginal smears of 140 postmenopausal women. (The last menstrual period had occurred at least one year before; all were above 35 years of age.) Our findings are the following:

In our series we have found 60% of postmenopausal patients with evidence of estrogenic stimulation. This finding agrees with the idea of the supplementary hormonal action of the adrenal cortex, as the ovaries are no longer functioning.

The distribution of the cytologic types as related to the age of women is similar to the reports of Luksh and Hannah Peters.

We wish to mention the fact that we have made an adaptation of our observations to the classification reported, in order to be able to make a comparison with the reports of the main speakers; but in our daily practice our results are classified with very good results with the excellent cytological classification of postmenopausal women of Pundel (1).

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HANS MUTH, Muenster in Westfalen, Germany:

As stated by Lang the definition of the word menopause is controversial. We understand that it means the last menstrual bleeding, whereas the first menstrual bleeding is called the menarche, and we call the period after this the climacteric. In agreement with the other authors we also recognize by means of cytology, signs of sometimes even subthreshold ovarian function, especially during the first several years of the climacteric. In the progressing senium, however, the atrophic smear type predominates as an expression of the ovarian inactivity.

We consider the adrenals as the main source of androgenic hormones after menopause. The frequent occurrence of androgenic type smears after surgical and radiological castration (24 to 39% according to our studies) speaks for this assumption.

The results of cytological examinations on 1099 women after physiological menopause have been condensed in the following Table:

(See Table on the next page)

Length of the Menopause	Type of Smear Atrophic	Intermediate	Proliferated	
1 to 5 years	11 (25%)	24 (56%)	8 (19%)	43
6 to 10 years	12 (33%)	19 (53%)	5 (14%)	36
11 to 20 years	24 (53%)	14 (31%)	7 (15.5%)	45
More than 21 years	9 (56%)	5 (31%)	2 (12.5%)	16
			Total	140

This chart belongs to Drs. Munguia and Franco.

PHYSIOLOGICAL CLIMACTERIC

DURATION OF CLIMACTERIC:	SMEAR TYPE:	Estrogenic	Decreased estrogenic activity	Mixed type	Androgenic	Atrophic	Totals
	1/2 - 1 year	24-18.3%	21-16%	21-16%	47-36%	18-13.7%	131
	to 2 yrs.	16-16%	9-9%	17-17%	36-36%	23-22%	101
	to 5 yrs.	9-3.8%	11-4.7%	32-14%	103-42.5%	83-35%	238
	to 10 yrs.	15-5%	8-2.7%	46-15.3%	91-31%	139-46%	299
	over 10 yrs.	6-1.8%	8-2.4%	42-13%	95-29%	179-54.2%	330
	Total and percentage	70-6.4%	57-5.2%	158-14.4%	372-33.8%	442-40.2%	1099

J. PAUL PUNDEL, Luxembourg, Luxembourg:

In a book published in 1950 (2), I adopted an interpretative classification for vaginal cytology in the menopause and after castration. However, as further studies showed that this period presents a very complex endocrinology, I have preferred now to return to a more descriptive classification (3):

1. superficial smear type
2. intermediate smear type
3. androgenic smear type
4. cytolytic smear type
5. parabasal smear type
6. completely atrophic smear type.

I think that this classification is nearly identical to that used by Montalvo Ruiz.

As was shown by all main speakers, there exists no specific smear picture which would characterize the postmenopause or castration. All the different smear types seen during these phases of life can be observed in other conditions. For the hormonal interpretation, we can relate, quite positively, the superficial smear type to a relatively strong estrogenic stimulation, the androgenic smear type to a predominantly androgenic stimulation, and the completely atrophic smear type to a more or less complete lack of hormonal stimulation. For the other smear types, it is in many cases very difficult, if not impossible, to relate them to a particular hormonal stimulation or to find out the different hormonal components, since in these smear types we can observe many variations of the individual smears, variations which affect the form of the cells, the type of desquamation and the higher number of smear types, but as so many smears show transitions from one type to the other, even with a more detailed classification, it would be difficult to group every smear in a precise manner. For this reason, I prefer to keep to a more sketchy classification. Furthermore, we know that the adrenal cortex plays an important part in the secretion of the hormones stimulating the vaginal epithelium after menopause and castration. And it is quite possible that the adrenal hormones are responsible for most of the smear pictures classified in types 2 to 5. As the adrenal cortex can secrete not only androgens but also estrogens and progesterone-like substances, and as the proportions of these hormones can vary from one patient to another, one can easily understand the variations and the complexity of the vaginal smears after menopause or castration, where it appears that the adrenal cortex attempts to supply the previous ovarian activity. Therefore, the classification of all types, as suggested by Wied (4) remains open for discussion.

I agree with Stoll that an atrophic smear should not be considered as an argument against a malignant tumor of the genital organs, as I also have seen cancers of the uterus and ovary in patients showing a completely atrophic vaginal smear. But in other patients, an abnormally high estrogenic smear for the age of the patient and the duration of the postmenopause has been the first suspicion of a tumor. Therefore, if one finds in an older patient an abnormally high estrogenic vaginal smear in the absence of estrogenic treatment, a complete gynecological examination including cervical smears should be recommended in order to exclude the presence of a genital tumor.

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JOSÉ R. del SOL, Madrid, Spain:

Of our last 2,000 cases, 253 were of women in menopause, excluding from this last figure those smears of women undergoing radiation.

Of the 253 menopausal smears, 33 cases (13.04%) were rejected, since they were beyond classification for the following reasons:

1. large amount of blood,
2. scarce cellular material,
3. strong inflammatory reaction,
4. malignant or pseudomalignant findings of such importance that their hormonal valuation was relegated to second term,
5. hormonal therapy previous to cytologic study.

For their classification we have followed the Wied criterion dividing them in four principal groups: 1) marked estrogen effect type, 2) slight estrogen effect type, 3) highly atrophic type, 4) non-atrophic type (there are no cellular signs of estrogen activity, but still they are not atrophic).

Marked estrogen effect type:
Found in 14 cases out of 220 = 6.36%. Of the 14 cases, three had clinical polyps and one had undergone mammary amputation seven years before. (The histologic diagnosis is unknown. The woman is now 62 years old.)

Slight estrogen effect type:
10 cases out of 220 = 4.54%.

Highly atrophic type:
76 cases our of 220 = 35.45%.

Non-atrophic type during menopause:
105 cases out of 220 = 47.72%.

In order to determine the relation between the findings of the smears and the time of menopause, the last menstruation date has been taken as the beginning of the menopause. The following are the results obtained:

	Less than 2 years	2-5 years	5-10 years	More than 10
1. M.E.E.	6 (42.85%)	1 (7.14%)	3 (21.42%)	4 (28.56%)
2. S.E.E.	4 (40%)	1 (10%)	----------	5 (50%)
3. A.M.T.	12 (15.38%)	15 (19.23%)	17 (21.79%)	34 (43.58%)
4. NA.M.T.★	35 (33.33%)	23 (21.90%)	21 (19.99%)	26 (24.75%)

★Corresponding to Wied's four groupings.

The S.E.E. group has a limited statistical value due to its scarce number. The high values of M.E.E. in women of less than 2 years postmenopause (42.35%) and of A.M.T. in women of more than 10 years post menopausal stand out.

CLOSING REMARKS

WARREN R. LANG

The discussants have emphasized some important points:

1. that there is no one type of "menopausal" smear,
2. that the presence of an estrogenic effect is per se no guarantee of the presence or absence of malignancy,
3. that an "estrogenic" effect is not necessarily hormonal much less necessarily ovarian in origin, and
4. that the cytologist who looks at a smear from a menopausal patient should be ever alert to the possibility of malignancy.

Zelma Kalnins mentions the fact that she would like to see "statistically evaluated data" relative to "cytologic pictures of sexually active and inactive menopausal women." This would truly advance our knowledge of cytology as would the use of statistical methods in medicine in general.

LUIS MONTALVO RUIZ:

In reply to Korte, regarding our description of the cytological pictures found during the menopause, we do not pretend to define a characteristic menopausal cytological pattern or anything like that with reference to the etiology and pathogeny of menopause. Doubtless, there are some individual factors that regulate such cytological patterns, but it is more a subject for internal medicine than cytology. In cytology we have to report our findings only to the clinician and he, with additional data (constitutional and individual characteristics) will develop his own opinions and therapy.

The other participants do not fundamentally disagree with us. Del Sol follows Wied's criteria; we follow Pundel's with slight modifications. We are very glad that in the present discussion Pundel now embraces a classification similar to ours. In substance, there are no discrepancies, since the type "marked estrogenic effect" would be equivalent to our "superficial type," and the type "slight estrogenic effect" resembles our "intermediate type;" the "highly atrophic," our "atrophic" and the "menopausal but not atrophic," our "parabasal." Although this classification does not mention the androgenic type, we include it as a variety of the intermediate type.

The contribution of Botella Llusia is interesting since it penetrates deeper and amplifies the endocrinological knowledge concerning menopause. We agree entirely with his observations.

HANNAH PETERS:

I would just like to underline two questions raised during the discussion:

1. The silent acceptance that estrogen stimulation often seen in smears after radiation to the ovaries is caused by hormonal activity of the adrenals. For this assumption there is as yet little experimental evidence. Perhaps the animal experiments done 20 years ago (1), which show that radiation causes luteinization and lutein tumors with hormonal effect, should stimulate further investigation to clarify this question. It has never been satisfactorily shown that radiation actually destroys all hormonal function of the ovary. The cause of estrogenic smears in radiated women therefore still remains unknown.

2. The suggestion made by Kalnins to obtain cytological information on sexually active and inactive menopausal women. This would be of great interest as it involves the question of whether or not sexual stimulation influences hormonal processes and if so, in what way.

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COMMENTS ARE INVITED
ABOUT ANY OF THE SUBJECTS TREATED
IN THE SYMPOSIA BY CORRESPONDENCE.

THE COMMENTS WILL BE PUBLISHED
IN THE SECTION "LETTERS TO THE EDITORS."

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ERRATUM

In Volume III, Number 3, page 415, the authors of the paper entitled RECURRENT CARCINOMA AND THE PRESENCE OF RADIATION CELL CHANGES were listed erroneously as Emmerich von Haam and Richard Albery. The correct names of the authors are Doctors Emmerich von Haam and Guenther Ceelen.